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EDITORIAL

QUIS CUSTODIET IPSOS CUSTODES?

THERE was a time when any man or woman, who really desired to enter one of the learned professions, could gain a place in a training school if he or she negotiated successfully the statutory preliminary educational requirements.

Such a statement may sound strange to those whose knowledge and information on such matters is limited to the period covered by the past decade. The explanation is a simple one—there were more places than applicants.

The schools jostled in their search for students, for the survival of an institution depended in no small measure upon the revenue derived from tuition fees. Although the means of entry was simple, the period of training was relatively, academically and economically, as severe and hazardous as it is to-day. There were few scholarships and in most cases the financial burden rested entirely upon the student or his parents.

In consequence, most of those who desired to enter a learned profession were imbued with a strong vocational urge and they were prepared to sacrifice many of the pleasures of life which were appropriate to their age and condition. These attributes were stimulated and encouraged by the willingness of parents to make great sacrifices in order to set their children's feet firmly on the road towards a chosen goal.

Now all this has suffered change. Success in the preliminary educational test does not lead automatically to a place in College but to a queue leading to a second test before a selection board. If the board looks with favour upon the candidate his troubles are not at an end, for he joins another queue to wait for a place to become available. In most cases he can cast aside the trials and tribulations of economic stringency or cramp during the undergraduate years,

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for a benevolent state thoughtfully undertakes financial responsibilities which were shouldered previously by parents.

The examination tests have also been modulated in their severity. Some subjects can be taken " one at a time " and on those occasions when it is required that the candidate shall attempt several subjects at the same time the lamp of reference glows with rays of comfort and hope. No longer is the candidate ushered into the presence of strangers who have no knowledge of his scholastic record or temperament, for his teachers are present and play a major role in the final assessment of his quality.

When one attempts to produce anything, be it animate or inanimate, the final product is the witness of the correctness and value of the methods used. Are the new methods for producing professional men and women better than the old? The môtif of the older method was an inward urge to follow a chosen career. Undoubtedly at the present time there are many young people who are moved in such manner, but their chances of attaining their hopes and ambitions depend in great measure upon their ability to surmount certain tests by examination whilst they are still at school. Their ordeal begins at the age of eleven and a failure to satisfy their examiners, even at such an early age, may condemn them for life. Little account seems to be taken of the fact that the " failure " at eleven may be the " success " of later years, and vice versa. Assessment of quality is in the hands of people who may be completely ignorant of the requirements of any career or profession other than their own.

The fortunate candidates under this system ultimately acquire sufficient certificates to satisfy the preliminary educational requirements of the universities and professions and then they are called to appear before a selection board of the university or college of their choice.

These selection boards have arisen because of a plethora of applicants for admission to the various schools. A selection board may be able to decide which candidates are likely to pass the examinations with the greatest ease, but can any selection board prognosticate which will make the best finished product and, by precept and service, be a credit to a profession?

Experience shows that this is by no means the case in the medical and veterinary professions. The professional examinations also demonstrate that even students of high preliminary educational standards show great variety in undergraduate attainment. Some step with ease over the hurdles in the pre-clinical period, only to fail miserably in the years when their studies are of a more vocational flavour and the reverse has been noted on many occasions.

Examinations are a necessary evil but it is tragic to think that there are many who are denied the opportunity to follow a career for which they are eminently suited vocationally because they are unable to produce evidence of competence in subjects which have little bearing on the life they long to lead. It is equally tragic that an undergraduate can be denied the right to qualify because of failures when he is within sight of his chosen goal. Many men who have achieved renown have not been successful examinees. The reason for failure does not always lie with the student.

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GENERAL ARTICLES

STUDIES ON THE PIG LUNGWORM (*METASTRONGYLUS* SPP.)

1.—Observations on natural infection in the pig in Great Britain

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Introduction

In 1952 a questionnaire was sent to some fifty veterinary surgeons in abattoirs, bacon factories and research laboratories in Great Britain to find out whether infection of pigs with *Metastrongylus* spp. is commonly met with. Most of the replies suggested that the infection is rare and some workers with much experience of post-mortem examination stated that they had never encountered pig lungworms. The few who stated that infection was common assessed its incidence as high as 25 per cent of all bacon pigs. The present paper presents the results of work carried out since 1952 to determine the incidence of lungworm infection in pigs sent for slaughter for bacon and also to see to what extent the disease can be detected on farms by the recognition of lungworm eggs in pig fæces and of the infective larvæ in earthworms, the intermediate host.

There are two published reports on the incidence of the disease in Great Britain, both based on surveys. Lewis (1926) reported that 50 per cent of 137 pigs examined in Mid-Wales were infected and that there was no seasonal variation in incidence. Robertson (1937) found lungworms in 13.08 per cent of 1,009 pigs examined in S.E. Scotland.

Observations

I.—Incidence of Lungworms in Pigs Slaughtered for Bacon.

A survey was conducted at three bacon factories. The procedure was to examine each pair of lungs as soon as the pluck was removed from the carcase. At the Cheshire factory it was possible to identify individual farm consignments of pigs by the numbers and to trace them to the district from which they had been collected, but no attempt was made to locate the actual farm from which each consignment came. Pigs at the second factory, which was in London, were traced only to the county of origin. At the third factory, in Hertfordshire, it was known only that the series of plucks examined came from pigs collected from a wide, unidentified area around the factory.

The lungs were examined by first inspecting and feeling for areas of emphysema and nodules and then incising as shown in Fig. 1. The caudal tip of the diaphragmatic lobe was cut off and the squeezed-cut surface examined for lungworms. An incision was then made parallel to the inferior border of the diaphragmatic lobe, and the cut surfaces inspected for lungworms. The apical and cardiac lobes were also incised and examined. The intermediate

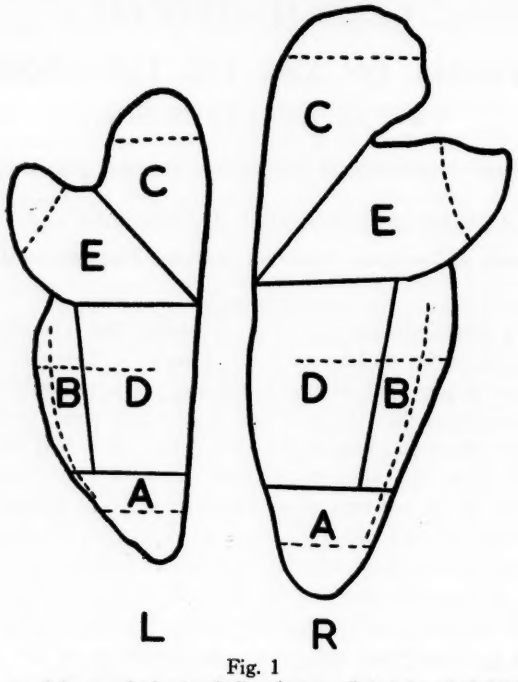

Fig. 1
Diagram of lungs of pig (excluding intermediate lobe of right lung) showing the approximate subdivision into areas and the sites of incision (dotted lines) used in the abattoir survey.

lobe (right lung) was not specially examined. The whole procedure—incising the various parts of the lung, squeezing the cut surfaces, and opening the bronchi with scissors—occupied about one and a half minutes for each pluck. Worms might well be overlooked by using so rapid a method, and a number of infected pigs might thus pass unrecognised. Experience with experimentally and naturally infected pigs has shown that at least an hour is required in the laboratory to examine thoroughly the lungs of a pig, including opening the very small bronchi where lungworms are so often located.

Cheshire Factory. An analysis of the findings in 890 pigs examined in this factory has been reported previously (Dunn, 1954). The final figures show that of 1,238 pigs examined over a period of 13 months during 1953 and 1954, a total of 225 (18.3 per cent) contained lungworms. Of the 224 consignments examined, 99 (44 per cent) contained infected pigs, which means that lungworms existed on nearly half of the farms from which pigs were sent to the factory. While some quite large infected consignments contained only a few pigs with worms (e.g., 1 of 18, 3 of 31), in other consignments almost every pig was infected (e.g., 10 of 14, 9 of 10). In the infected consignments 31 per cent of the individual animals were infected.

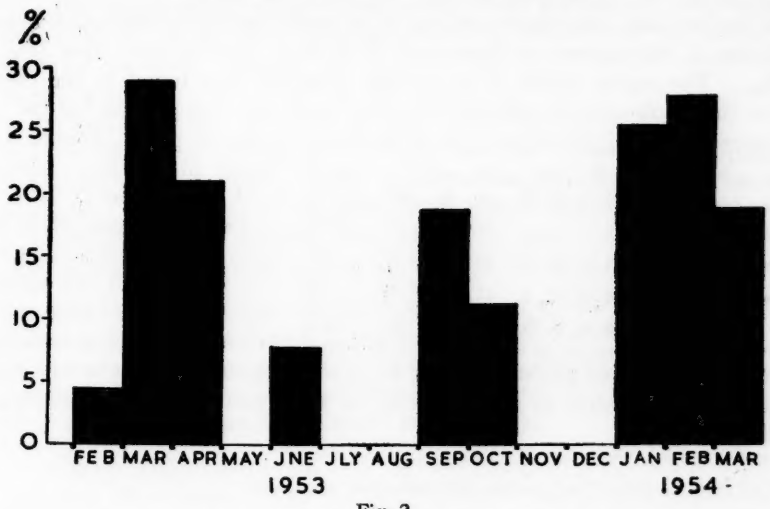

Fig. 2
Incidence of lungworm infection in bacon pigs at Cheshire factory.

The figures for incidence in pigs collected from different districts showed no obvious difference, but the area was so large and the districts so numerous that no conclusion as to regional incidence could be drawn, except to record that infected pigs were detected in consignments from 33 of the 40 districts from which pigs came during the days of the survey. Nine visits were paid to the factory over a period of 13 months and 120-180 pigs were examined on most occasions. The incidence of infection ranged from 4.5 per cent in February, 1953, to as high as 28 to 29 per cent in March, 1953, and February, 1954 (Fig. 2).

London Factory. On two successive days in November, 1954, a total of 366 plucks were examined and 96 (26 per cent) were found to be infected. The pigs came from eight counties in S.E. England (Bucks, Cambs, Essex, Hants, Herts, Hunts, Kent and Surrey) and there were infected pigs in one or more consignments from each county.

Hertfordshire Factory. The lungs from 118 pigs were examined on one day in September, 1953, and 32 (27 per cent) were found to be infected. The exact origin of the pigs was not known but the factory served a wide area.

The incidence of lungworms was very similar in the three factories, although tending to be higher in S.E. England than in the North-West. Infection was found in pigs from at least 11 counties in England, as well as from parts of North Wales and in a consignment from the Isle of Man. Of the total of 1,722 pigs examined at the three factories, 353 were infected, an incidence of 20.5 per cent.

II.—Distribution of Lungworms in the Lungs of Bacon Pigs.

In many pigs worms were present in only small numbers (10 to 50) and were most often located in the caudal tip of the diaphragmatic lobes. To see

how the parasites were distributed the lungs were examined so that records were available of the presence of lungworms in each of five different areas (Fig. 1, A-E). The results (Table 1) show that parasites were found in the caudal tip of the diaphragmatic lobe and nowhere else in 247 (70 per cent) of the 353 infected pigs. Worms were found in this area, together with areas B or D, or both, in another 95 animals, so that area A was involved in 341 (97 per cent) of 353 infected pigs. Worms were never found in the apical or cardiac lobes, and the diaphragmatic lobe (A, B. D) was thus the only lobe in which the parasites were detected in bacon pigs by the rapid method of examination used in the survey.

TABLE 1. Location of lungworms in the lungs of bacon pigs.

FACTORY	AREAS AFFECTED (Fig.1.)									TOTAL
	A	B	C	D	E	AB	AD	BD	ABD	
Cheshire	167	1	0	9	0	4	22	1	21	225
London	64	0	0	0	0	13	10	0	9	96
Herts	16	0	0	0	0	7	1	0	8	32
Total	247	1	0	9	0	24	33	1	38	353

III.—Detection of Lungworm Infection on the Farm.

During 1953 a survey was made of a number of farms near the laboratory to see whether lungworm infection could be detected by examining the fæces of pigs for lungworm eggs and also to obtain an indication of the ease with which lungworm larvæ could be found in earthworms on infected premises. At this time sodium chloride was being used for flotation of eggs. The subsequent finding that saturated salt solution reveals only a small proportion of lungworm eggs when used as a flotation fluid (Dunn and White, 1954) means that the method may well have missed infected pigs, particularly those with low egg counts.

Visits were made to 19 farms and smallholdings where pigs were kept. On ten of the farms the pigs were never allowed out and were thus unlikely to become infected with lungworms, save by contact with infected sods or manure containing earthworms. No lungworm eggs were found in samples of fæces from pigs on these ten farms. On the other nine farms earthworms were collected from the pastures to which the pigs had access, as well as samples of fæces from the pigs.

The earthworm species collected included *Allolobophora terrestris, A. caliginosa, A. chlorotica, Eisenia rosea, Lumbricus terrestris* and *L. rubellus.* All six species have been found to be susceptible to experimental infection with lungworms (Dunn, 1955). The species of earthworm most easily infected experimentally, *Eisenia fœtida,* which is a common inhabitant of dung and compost heaps, was not found in the pig paddocks.

Naturally infected earthworms were found on three of the nine farms where pigs were allowed out to grass. Only a few larvæ were found in each worm, usually 3 to 7 and never more than 20, and only a small proportion of all the worms examined was found to be infected. In contrast, experimentally infected *E. fœtida* usually contained at least 100 larvæ and every earthworm exposed to lungworm eggs became infected.

The only samples of pig fæces which were positive for lungworm eggs were from one of the three farms where infected earthworms were found. The stock on this farm comprised two breeding sows and four five-month-old store pigs, two of which had been recently bought in. All the animals spent most of the day in a grass paddock and were housed at night. Using salt solution and the McMaster slide method of egg counting, lungworm eggs were found in samples of fæces from both the home-bred and bought-in store pigs on two occasions. The counts ranged from 100 to 2,200 e.p.g. in individual samples. None of the animals coughed when at rest or when chased around. Fæces samples from the two sows showed 50 e.p.g. and 0 e.p.g. respectively.

The later finding that saturated salt solution is unreliable for flotation of lungworm eggs was confirmed by the fact that detection of infected earthworms on these farms was a more sensitive method of diagnosis of lungworm than fæces examination since whereas infected earthworms were found on three farms, fæces examination revealed infection on only one.

During 1952 and 1953 some hundreds of samples of pig fæces were examined for helminth eggs by salt flotation in order to study the incidence of infection with *Œsophagostomum* and *Hyostrongylus*. The method was found to be very satisfactory for eggs of these species. During this time lungworm eggs were seen on only one occasion, yet at the same time the survey at bacon factories showed an incidence of infection of about 20 per cent. Since using saturated magnesium sulphate (S.G.1.285) it has been possible to detect lungworm eggs with ease and accuracy. Samples of fæces from naturally and experimentally infected pigs have given weekly counts that were so reliable that on more than one occasion they have served to detect errors in labelling samples. The method used was to shake a two-gramme sample of fæces with about 30 ml. of saturated magnesium sulphate solution ($MgSO_4$, $7H_2O$), pass through a wire sieve of 44 meshes to the inch, distribute in two 15 ml. centrifuge tubes, top up with the solution, cover with a coverslip, and centrifuge at 1,500 r.p.m. for three minutes. The technique adopted was to count the eggs in the first coverslip from each tube and then to top up and centrifuge the tubes a second time and examine the second pair of coverslips. With this method it was possible to detect counts as low as 2 to 5 e.p.g. week after week and the technique was found equally reliable for *Œsophagostomum*, *Hyostrongylus* and *Trichuris* eggs. The eggs commonly found in pig fæces are illustrated in Figs. 3 and 4 which were prepared from routine spins using saturated $MgSO_4$.

In April, 1953, a series of samples of rectal contents had been collected from 70 bacon pigs at the Cheshire factory which were part of a larger series being examined at the same time for adult lungworms by inspection of the lungs. Sixteen of the 70 pigs showed adult lungworms and yet only a single lungworm egg was found in all the samples when they were examined by both the McMaster and direct flotation methods, using saturated NaCl. It was this failure to find lungworm eggs in the fæces of infected bacon pigs and the finding

that egg counts in most infected adult pigs are low (2 to 50 e.p.g.) that caused us to change to magnesium sulphate for flotation.

Since the autumn of 1954 an investigation into the incidence of lungworms has been in progress on a farm (Farm A) near the laboratory where there are some 40 sows and 300 fattening pigs. Losses from pneumonia are fairly common and the clinical history is typical of the virus pneumonia syndrome, complicated by clinical breakdowns due to secondary bacterial infection. No lungworms had been seen at any time although a number of lungs had been examined post-mortem. The results of our investigation show that at least half of the adult and growing animals on the farm harbour lungworms and almost all the pasture plots to which pigs have access contain infected earthworms. So far, for instance, mainly from examination of single fæcal samples, lungworm eggs have been found in 1 of 2 boars, 7 of 15 sows and 15 of 28 litters of fattening pigs. Four of six young pigs dying from pneumonia between the ages of two and four months have shown adult lungworms. Three were fairly heavy infections (150 to 180 worms), and one was light (20 worms). Although the worms were most numerous in the diaphragmatic lobe, especially at its posterior end, as they were in bacon pigs, they were also found in the cardiac lobe in two pigs and in the apical lobe in one. (It will be recalled that lungworms were never found outside the diaphragmatic lobe in routine examinations in bacon pigs.) Furthermore, in histological sections of the apical and cardiac lobes numerous lungworm eggs containing fully-developed larvæ were seen.

A total of 229 earthworms was collected from the farm, identified, and examined for lungworm larvæ. The incidence of infection in the various species is shown in Table 2. Typical lungworm larvæ were found in the crop (Fig. 5) in earthworms from each of the three paddocks used for gilts and pregnant sows and from 14 of the 18 grass runs with portable houses in which sows farrowed and reared their litters to weaning. Lungworm larvæ were found in 21 per cent of the earthworms. Most of the infections were light (under 10 larvæ), and only two of the 48 positive earthworms contained more than 100 larvæ (250 and 380 respectively).

TABLE 2. Incidence of infected earthworms on Farm A.

SPECIES	EXAMINED	INFECTED	NOT INFECTED
Allolibophora terrestris	99	29	70
A. caliginosa	59	8	51
Lumbricus spp	30	9	21
Eisenia rosea	3	1	2
Octolasium cyaneum	1	0	1
Immature and unidentified	37	1	36
TOTAL	229	48	181

There was thus evidence on this farm of a very widespread infection with lungworms, in which litters became infected during the suckling period which they spent on grass runs contaminated by the fæces of the sows and of previous litters. After their transfer to fattening houses at weaning time the store pigs caused no further contamination of the pasture, except indirectly through the

PLATE I

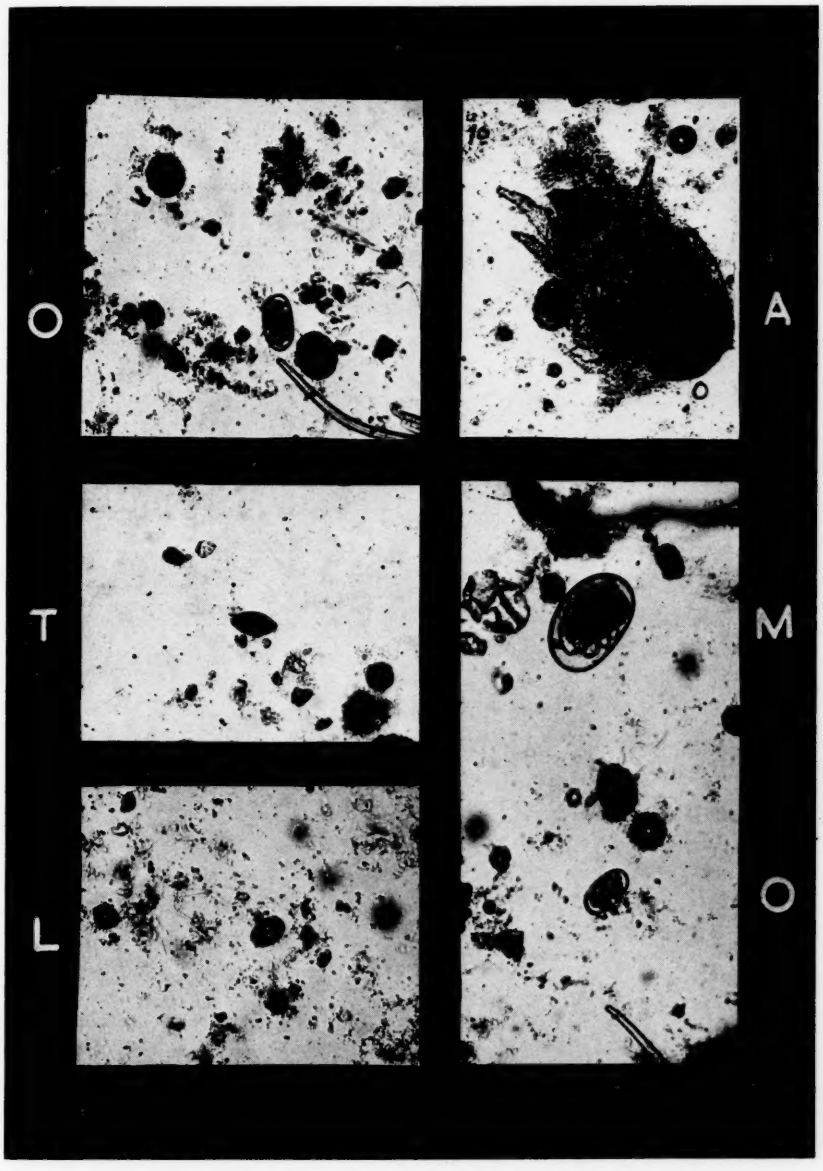

Fig. 3
Low power composite picture of eggs commonly found in pig fæces. A: *Ascaris;* L: lungworm; M: mite (mite itself top right); O: *Œsophagostomum* or *Hyostrongylus;* T: *Trichuris.* x 116.

(*Article by Dunn, Gentles and White, page* 271)

PLATE II

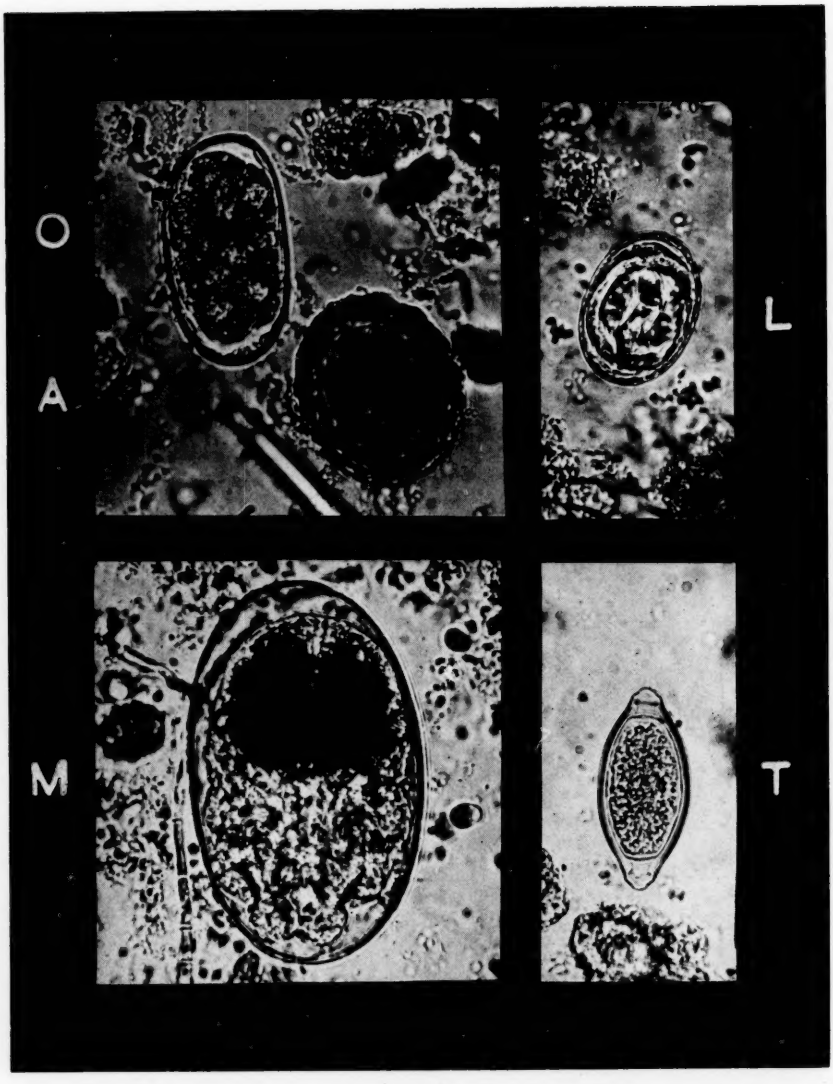

Fig. 4

High power composite picture of eggs commonly found in pig faeces. A: *Ascaris;* L: lungworm; M: mite; O: *Œsophagostomum* or *Hystrongylus;* T: *Trichuris.* x 470.

(*Article by Dunn, Gentles and White, page* 271)

PLATE III

Fig. 5
Third stage (infective) lungworm larvæ in wall of crop of earthworm (*Allolobophora terrestris*) from naturally infected pig pasture. Fresh preparation, unstained. x 73.

Fig. 6
Fifth stage larva of *M. apri* in lung of pig five days after experimental infection. Larva is surrounded by eosinophils. H.E. x 116.

Fig. 7
Section of small bronchus containing mature lungworms (3 ♀, 1 ♂) and showing chronic bronchitis and peribronchial lymphoid hyperplasia. Naturally infected bacon pig. H.E. x 34.

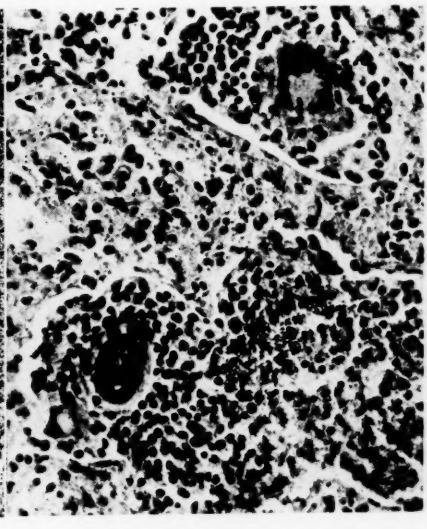

Fig 8
Eosinophil and giant cell reaction excited in lung alveoli by lungworm eggs in naturally infected pig. One alveolus (top right) contains a giant cell, another (bottom left) an embryonated egg. H.E. x 300.

(*Article by Dunn, Gentles and White, page* 271)

PLATE IV

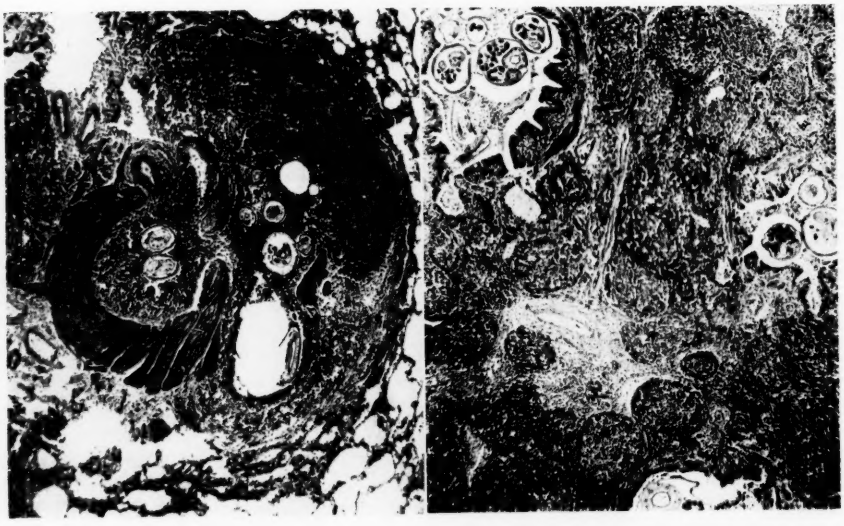

Fig. 9
Section of lung of bacon pig showing adult lungworms impacted in bronchiole which shows great thickening of its muscular coat and hyperplasia of peribronchiolar lymphoid tissue. H.E. x. 30.

Fig. 10
Portion of lymphoid nodule (5 mm. diameter) in lung of pig showing follicular structure and relationship to bronchioles containing lungworms. H.E. x 30.

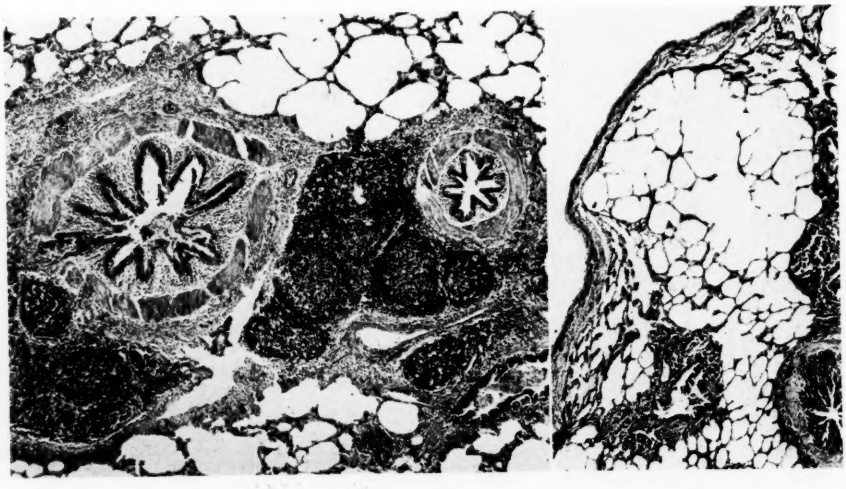

Fig. 11
Hypertrophy of bronchiolar muscle and hyperplasia of peribronchiolar lymphoid tissue due to lungworms. No parasites in section but lung contained 926 adult lungworms. H.E. x 36.

Fig. 12
Low power view of ventral border of diaphragmatic lobe of pig lung showing vesicular emphysema associated with impaction of bronchioles with lungworms. No parasites in section but 54 lungworms found in the lungs. H.E. x 30.

(*Article by Dunn, Gentles and White, page* 271)

use of their manure as fertiliser, but the sows kept the infection going in the paddocks and runs.

While using NaCl flotation in 1953 for routine weekly fæcal egg counts on two sows and their litters kept at this Veterinary Field Station (Farm B) no evidence of lungworm eggs was obtained, yet it was later found that half of one litter and all of a second litter were infected when they reached the bacon factory. In the summer of 1953 a four-month-old pig had to be destroyed because of injuries inflicted by fighting. Adult lungworms were found, and a change to $MgSO_4$ instead of NaCl for flotation then revealed low levels of lungworm eggs in the fæces of most of the remaining animals every week until they were sent for bacon.

The use of $MgSO_4$ gave the results that were to be expected and proved at least as sensitive an indicator of infection on premises as did the examination of earthworms. It was sometimes even more sensitive, since three successive batches of 30 to 50 earthworms taken from the plot at the Field Station (Farm B), where the infected pigs ran, had to be examined before infective larvæ could be found, whereas weekly fæces examinations never failed to show lungworm eggs, although the counts were often as low as 0.5 e.p.g. for several successive weeks. The incidence of infection in earthworms on the plot used by pigs increased subsequently to 27 per cent (Table 3).

TABLE 3. Incidence of infected earthworms on Farm B.

SPECIES	EXAMINED	INFECTED	NOT INFECTED
Allolobophora terrestris	14	4	10
A. caliginosa	7	2	5
A. chlorotica	4	1	3
Lumbricus terrestris	1	0	1
TOTAL	26	7	19

IV.—Pathological Changes Caused by Lungworms.

In bacon pigs the lesions are usually inconspicuous. Wedge-shaped lobular areas of vesicular emphysema are seen along the ventral border of the diaphragmatic lobe near and at its caudal end. There is no consolidation in these areas. Associated with the emphysematous areas there are often a few firm greyish nodules, usually 1 to 3 mm. in diameter. The lungworms are found lying inside the thickened and dilated bronchi and bronchioles within the emphysematous areas. Sometimes single lungworms are found completely blocking bronchioles just large enough to contain them and situated almost at the lung border. Larger bronchi, situated more deeply within the lung tissue, are often blocked by a dozen or more adult worms. The main bronchi seldom contain lungworms and it is necessary to open the smallest ones, near the lung surface and borders, if the relatively few lungworms present in most infected pigs of bacon weight are to be detected. The histological changes are illustrated in Figs. 6 to 12 and include chronic bronchitis (Fig. 7) with mucoid metaplasia of the bronchial epithelium, enormous hypertrophy of the bronchial musculature and of the muscle fibres in the walls of bronchioles (Figs. 9 and 11), alveolar ducts

and alveoli, and peribronchial lymphoid hyperplasia (Fig. 11) with the formation of lymphoid nodules which sometimes reveal their peribronchial origin by showing a central cavity occupied by parasites (Fig. 10). Eosinophils are numerous in most lesions. The lymphoid nodules seen in the lung closely resemble those described in the liver of the pig some years ago by one of the authors (Oldham and White, 1941) and ascribed to *Ascaris* migration. Similar liver lesions (milk spots) with eosinophil infiltration of the interlobular tissue and periportal lymphoid nodules, were seen in some of the pigs infected experimentally with lungworms and free from other helminths (Dunn, 1955). It seems that "milk spot" liver in the pig can thus be caused by the accidental migration of the larvæ of *Metastrongylus* spp. through the liver as well as by *Ascaris* larvæ. The lymphoid nodules in the lungs are a feature of lungworm infections of long standing and are not seen in recent infections in young pigs.

In younger pigs there are other changes, including the initial lesion caused by the fifth stage larva arriving in the lung (Fig. 6) and a parasitic pneumonia with giant cell formation associated with the liberation into the alveoli of large numbers of eggs (Fig. 8) from worms that have just reached sexual maturity. The more severe and widespread lesions may be of some importance in relation to the pathogenesis of pneumonia in young pigs, since they occur in animals before or at about weaning time and may develop in any part of the lung, including the anterior and cardiac lobes which are the predilection site for the pneumonic lesions caused by viruses and bacteria.

Discussion

Much lungworm infection in bacon pigs is probably missed because of the inconspicuous lesions which usually characterise the infection. At this age infections are usually light and in most cases would be overlooked unless the caudal end of the diaphragmatic lobe is examined, the finer bronchi opened, and the cut surfaces squeezed to express the worms. Those who have once recognised the condition in bacon factories are in no doubt about its high incidence. In younger pigs the infection is also often missed. We have seen heavy infections in several pigs with lesions of pneumonia which had been attributed to viral and secondary bacterial infection and in which no lungworms had been noticed. In the presence of gross pneumonic lesions in the anterior and cardiac lobes there is a tendency to overlook lungworms.

Since almost 20 per cent of bacon pigs in some eleven counties of Britain were found to be infected with lungworms it is probable that an even higher percentage of young pigs is affected, for infection is most likely to be acquired when the animals are young, the time when they most often have access to pasture. The observations we have made on young pigs support the view that the incidence of infection on farms carrying lungworms is high. At this Field Station, for instance, we have found an unsuspected incidence of infection of 50 to 100 per cent in six litters during recent months, although the ground is not heavily infected and the pigs are on it for only a few weeks. A period of one week on a lightly-infected grass run was sufficient to produce light infections

in all of four pigs which had just been weaned. On the large pig farm mentioned earlier (Farm A) infection was detected in 15 of 28 litters, in most cases by examining only a single sample of mixed fæces. The figures obtained at the bacon factories showed infection in nearly half of all consignments of pigs, which means that lungworms were present on at least that proportion of farms. More detailed inspection would show the incidence to be higher, for we have been able to find lungworms by careful search in lungs which were negative to the rapid routine procedure. On heavily infected premises it is likely that most young pigs would acquire the infection.

The fact that lungworms in most bacon pigs were confined almost entirely to the caudal part of the diaphragmatic lobe seemed at first very surprising, for it is established that lungworm larvæ reach the lung by the pulmonary artery, having first moulted twice in rapid succession in the mesenteric lymph nodes and reached the vena cava by way of the thoracic duct (Schwartz and Alicata, 1934). The almost exclusive involvement of the diaphragmatic lobe in the bacon pig might have several explanations: (1) Seeding of this part of the lung with larvæ, to the almost complete exclusion of the other parts; (2) failure of the worms to develop to the mature stage, except in the diaphragmatic lobe; (3) expulsion of most adult worms, alive or dead, from the lung of the older pig, except from the posterior part of the diaphragmatic lobe. The first two possibilities are unlikely since we have found mature lungworms throughout the lung in a number of young animals, naturally or experimentally infected, and also in a number of adult animals. The third explanation is the most likely, but it is also possible that there may be some amount of movement of adult worms from one part of the lung to another, such as might be caused by the expulsion of worms from bronchi by coughing and their subsequent aspiration into other bronchi.

It is probable that the eventual localisation of most lungworms in the caudal tip of the diaphragmatic lobe in adult pigs represents a residual infection which at one time affected the whole lung. This does not mean that particular areas of the lung may not be more suitable than others for their development for there remains the question of why only the diaphragmatic lobe retains the infection. A possible explanation is that coughing is more easily able to expel live or dead lungworms from the bronchi in the apical and cardiac lobes than from the more caudal part of the diaphragmatic lobe, where lodgment in the terminal bronchi and the bronchioles provides a more secure and permanent home for the worms. In pigs which are not seriously affected by the initial widespread development of mature worms and the large crop of eggs they produce there is a reaction in the form of coughing which probably gets rid of many of the parasites, and a tissue reaction which deals with the helminth eggs as foreign bodies and destroys or removes them. In most pigs the end result is a light residual infection which is confined to the caudal tips of the lungs. Sometimes a heavier infection persists, and there are also deaths among young pigs due to heavy infections. It is also not unlikely that some of the losses from bacterial and viral pneumonia in pigs are in part due to lungworm

infection producing damage which favours the establishment of other infective agents.

Some support for the view that adult lungworms may be expelled from the anterior lobes was provided from some recent observations on the lungs of young pigs with fairly heavy natural infections. The numbers of worms found by dissection in each lobe were recorded separately and histological sections were prepared from each lobe. Adult lungworms were found in the cardiac and diaphragmatic lobes but none of the females contained fully-developed embryonated eggs. Fresh films and histological sections showed embryonated eggs were also present in the apical lobes although no adult worms were found in them. A possible explanation is that the female worms which produced the embryonated eggs found in the apical lobes had already been expelled by coughing. The naturally infected pigs mentioned above died of pneumonia, and the clinical picture on the farm was considered typical of virus pneumonia, with secondary breakdown caused by bacterial infection. The lungs were extensively consolidated and there was pleurisy and widespread suppuration. The lungworms would probably not have been noticed.

Whether infection in bacon pigs is of economic importance is not known. Infection is found equally in good and poor bacon pigs.

Young pigs which are on infected ground during the suckling period acquire repeated doses of infected earthworms, which they actively search for and greedily eat. When a box of soil containing 56 earthworms, including the odorous *Eisenia fœtida*, was placed in a grass pen with a litter of five 14-week-old pigs, every worm had been found and eaten within 20 minutes. Pigs may sometimes be more successful in finding infected earthworms than a man with a spade, for whereas young pigs regularly developed lungworm infection after only a few weeks on a plot at the Field Station (Farm B), three successive batches of earthworms had to be examined before a single lungworm larva was found.

Summary

1. Among 1,722 pigs examined for lungworms (*Metastrongylus* spp.) at three bacon factories (Cheshire, London and Hertfordshire) the incidence of infection was 20.5 per cent. The pigs came from farms distributed widely throughout eleven counties of England and part of North Wales.

2. In the Cheshire bacon factory 99 (44 per cent) of 224 consignments (1,238 pigs) contained one or more infected pigs, and infection was found among consignments from 33 of 40 different districts in N.W. England and N. Wales as well as in a consignment from the Isle of Man.

3. In bacon pigs the parasites were mainly confined to the diaphragmatic lobe, and usually to its caudal end. The lesions appeared as lobular areas of emphysema, often accompanied by greyish lymphoid nodules. Lungworms were present in only small numbers in most infected animals.

4. Examination of fæces from pigs on nine farms revealed lungworm eggs on only one occasion when saturated NaCl was used for flotation, whereas infection was found in earthworms on three of the farms. The failure of saturated NaCl as a flotation fluid for lungworm eggs is discussed and the advantages of saturated magnesium sulphate solution ($MgSO_4$, $7H_2O$) are mentioned.

5 Examples are given which show that a high proportion of the pigs on infected farms harbour lungworms. Infective larvæ can usually be detected with ease in earthworms on such farms.

6. The histopathological changes produced by lungworms in the lungs of the pig are described briefly and illustrated.

7. The possible importance of lungworm infection of pigs is discussed and it is suggested that it may be greatest in young pigs, at or shortly after weaning. The infection in adult pigs is probably a residual one.

Acknowledgments

We are grateful to the managers of the three bacon factories, especially the one in Cheshire, for the facilities and hospitality extended to us. We also wish to thank those who replied to the questionnaire and those who kindly provided infected material. Dr. N. Tebble, of the British Museum (Natural History) kindly checked the identity of a number of earthworms, and we wish to thank Messrs. Flemming and Irvine of the Department of Zoology, University of Liverpool, for their valuable assistance in preparing some of the photographs.

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REFERENCES

Dunn, Diana Rowan (1954) : *Trans. R. Soc. trop. med. Hyg., 9.*
Dunn, Diana Rowan (1955) : *Brit. vet. J., 111,* 97.
Dunn, Diana Rowan, and White, E. G. (1954) : *Nature, 174,* 1193.
Lewis, E. A. (1926) : *J. Helm., 4,* 123.
Oldham, J. N., and White, E. G. (1944) : *J. comp. Path., 54,* 1.
Robertson, D. (1937) : *Scottish J. Agric., 20,* 1.
Schwartz, B., and Alicata, J. (1934) : *U.S. Dept. Agric. Tech. Bull.,* 456, p. 41.

SURVEY OF LABORATORY ANIMALS IN GREAT BRITAIN

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Part I.—The Survey

In January, 1953, a questionnaire was sent to all laboratories in Great Britain where animals were, or were thought to be, used. With each questionnaire went a covering letter, explaining briefly the purpose of this inquisition, and a postcard for convenient reply, stating whether or not the laboratory intended to return a completed questionnaire.

This was not the first time that an attempt had been made to survey the production, supply and uses of laboratory animals in this country. For example, a committee on the supply of experimental animals set up in 1943 had circulated questionnaires to users and breeders, and gained therefrom some knowledge about the problem, and from 1947 to 1949 the Laboratory Animals Bureau had followed up this work by the same method: but the scope of these earlier inquiries was limited. The purpose of the present inquiry was to obtain as much useful and detailed information as possible about the supply and use of experimental animals. The questionnaire referred to the year 1952.

Great care was taken to compile a complete list of laboratories, and it is unlikely that any laboratory using an appreciable number of animals was omitted from the circulation list. In the case of some universities and one or two other large organisations, a number of questionnaires was sent to the registrar or secretary, at his request, for internal distribution. In nearly every case more forms were sent than were required, which makes the percentage response look lower than it was. In one case, moreover, not one of a whole batch of forms was ever heard of again, and it must be presumed that they were never distributed.

Information was sought concerning all vertebrates, but in the analysis fish have been excluded. Some uncertainty was apparent about whether reptiles and amphibia on the one hand, and farm animals on the other, should have been included in replies. It was intended that they should be, and for the most part they probably have been, but in certain instances it is suspected that they have been omitted.

In the design of the questionnaire, a compromise was struck between the sort of questions inviting an indefinite and prosy reply, incapable of objective analysis, and the one-word or -number reply, which, while simple to analyse mechanically, leads to enormous multiplication of questions.

Grouping of Laboratories

In the analysis all laboratories have been placed in one of three groups, as follows:—

Group I: Laboratories where the main emphasis is on the diagnosis of disease in man and animals: for example, public health, hospital, and veterinary investigation laboratories.

Group II: Laboratories chiefly engaged in research, and also teaching: for example, universities and research institutes.

Group III: Laboratories belonging to commercial organisations, where the work is directly connected with the production or testing of drugs and other therapeutic substances.

There was bound to be some overlap between these groups, but this did not provide any great difficulty.

Response to Questionnaire

Table 1 shows the result of sending out 675 questionnaires.

Table 1

The number of questionnaires sent out and returned. Figures in brackets are the percentage of the total.

	Group I	Group II	Group III	Total
Positive replies	182 (27.0)	147 (21.8)	26 (3.9)	355 (52.7)
Negative replies	67 (9.9)	64 (9.5)	18 (2.6)	149 (22.0)
Replies promised but not received	18 (2.7)	34 (5.0)	9 (1.3)	61 (9.0)
No news at all	34 (5.0)	69 (10.2)	7 (1.1)	110 (16.3)
Total questionnaires sent out	301 (44.6)	314 (46.5)	60 (8.9)	675 (100)

The positive replies were those that gave information sufficiently detailed to be analysed, and on which this survey is therefore based.

Negative replies included those who had declined for any reason to give information, who had no animals to return, or whose replies were so incomplete that they could not be analysed (but see note on Table 2).

A few laboratories promised to send replies, but, in spite of reminders, never did so.

Lastly, there were those who never returned the original postcard, never replied to reminders, and of course never sent in their questionnaires. The figure for this is not so bad as it looks, for it includes those questionnaires sent out in bulk and not distributed; any which may have been misdirected; duplicates, where two authorities share the same laboratory, e.g., a public health laboratory director and a hospital pathologist; and at least some sent to laboratories where animals were no longer used.

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LAB/U/21
January, 1953

On completion please return to:-
Laboratory Animals Bureau,
M.R.C. Laboratories,
Holly Hill Hampstead, N.W.3.

LABORATORY ANIMALS BUREAU
QUESTIONNAIRE

NOTES:

(i) Approximate numbers will in most cases suffice. Even an estimate is useful.
(ii) Where answers cannot be given, this fact should be indicated, e.g. by stating "not known". This will prevent the form being sent back for further information.
(iii) Please err on the side of fullness in giving details, using a separate sheet of paper if necessary. The information will be regarded as confidential.

QUESTION 1 - Please give the following details in respect of animals used in 1952:-

Column "A" SPECIES, Type or Strain	Column "B" SOURCES. State "own breeding" or, if from another laboratory or breeder or dealer, please name.	ColumnC"C" NUMBERS from each source.	Column "D" PURPOSES for which used. Please give details as far as possible.	Column "E" COST per animal.

QUESTION 2.

(a) If you breed your own animals, what are your reasons for doing so, rather than obtaining them from elsewhere?

(b) If you obtain your animals from elsewhere, what are your reasons for choosing your particular sources?

QUESTION 3.

What incidental losses, due to intercurrent disease and other causes, have you sustained?
What have such losses been due to?
Please give details, including species and numbers lost, time of year, etc.

QUESTION 4.

In what ways do any of the animals you have been using fall short of your requirements?

QUESTION 5.

Carter et al ("Cancer Research, 1952, 12 No.8,602") referring to mice, define the term "inbred strain" as follows:-

"A strain shall be regarded as inbred when it has been mated brother X sister for twenty or more consecutive generations. Parent x offspring matings may be substituted for brother X sister matings, provided that in the case of consecutive parent X offspring matings the mating in each case is to the younger of the two parents."

Do you use inbred strains, as so defined, of any species? If so, of what species, and for what purposes (if not already indicated under Question 1)?

Would a lesser degree of genetic uniformity suffice, e.g. cousin mating, random mating in close population? Please differentiate by species and purposes.

QUESTION 6.

Are you maintaining strains of any species which are inbred, or which show a considerable degree of constancy or uniformity?

If so, will you please list them.

QUESTION 7.

(a) In obtaining your requirements of laboratory animals, has the Laboratory Animals Bureau been of any assistance to you?

Have you any suggestions for improving its services?

(b) If you have had occasion to use guinea pigs, mice, or rabbits accredited by the Laboratory Animals Bureau, have you found them to be superior to non-accredited animals?

(c) If you do not already receive the L.A.B. half-yearly News Letter, would you like to be placed on the distribution list?

QUESTION 8.

Please add any further remarks which you think may be of help.

DATE ..... SIGNATURE .....LABORATORY .....

With few exceptions the questionnaire was carefully answered.

The Questionnaire

The questionnaire was a sheet of paper 13 in. by 16 in., printed on both sides. There were eight questions, and some of them were in more than one part (see page 284). With few exceptions it was carefully answered, and the information provided was to the point. In one important respect only did the questionnaire fail to elicit useful information, namely in the cost of animals. In most cases this column (column E, Question 1) was left blank; in others it was filled in in such a way as to indicate that the words "cost per animal" might be interpreted in different ways. No attempt, therefore, was made to analyse the answers to this part of Question 1.

Question 1

Table 2 shows the number of laboratories using different types of animals. In this case, and this alone, have been included laboratories that gave the species used, but no numbers of each; their questionnaires had therefore to be excluded

Table 2

Number of laboratories using different types of animals. +

Guinea pigs	283
Hamsters	30
Mice	183
Rabbits	218
Rats	122
Cats	42
Dogs	35
Ferrets	12
Cows	6
Goats	8
Horses	3
Pigs	3
Sheep	5
Primates	17
Other mammals	9
Birds	41
Reptiles & amphibia	26

+ This table includes those laboratories that indicated the species they used, but did not give any figures. In all other tables they have been ignored, which accounts for the discrepancy with Table 5.

from the rest of the inquiry. Hence the apparent discrepancy between Table 2 and Table 5.

Table 3 shows the over-all figures of animals of all species used in laboratories of the three groups. "Other mammals" include hedgehogs, shrews, voles and some uncommon rodents.

Table 3

Numbers of animals of various kinds used in different groups of laboratories.

	Grp. I	Grp. II	Grp. III	TOTAL		PERCENTAGE
Guinea pigs	132,058	60,926	6,591		199,575	11.4
Hamsters	144	2,452	2,050	4,646		
Mice	190,403	514,745	475,692		1,180,840	67.8
Rabbits	10,328	19,844	2,996		33,168	1.9
Rats	14,755	110,106	125,077		249,938	14.4
Cats	300	4,717	436	5,453		
Dogs	183	7,271	56	7,510		
Ferrets	33	3,232	-	3,265		
Cows	11	159	-	170		
Goats	50	81	-	131		
Horses	36	17	-	53		
Pigs	-	24	-	24		
Sheep	31	138	-	169		
Primates	804	147	50	1,001		
Other mammals	-	1,228	-	1,228		
Canaries	-	108	-	108		2.5
Chickens	210	34,561	7,946		42,717	
Pigeons	555	586	464	1,605		
Reptiles	-	82	-	82		
Amphibia	829	9,062	100	9,991		
					35,436	2.0
Total	350,730	769,486	621,458		1,741,674	100

Five species (mice, rats, guinea pigs, rabbits and chickens) were each used in numbers greater than one per cent of the total, and the remainder added together accounted for no more than two per cent. Mice provided over two thirds of the total, a rather lower proportion than in previous estimates (Lane-Petter, 1953*a*) but still of the same order of preponderance. In fact, this discrepancy can be largely accounted for by the fact that one laboratory using very large numbers of mice failed to return a questionnaire.

Table 4 shows the average number of animals used per laboratory within each of the three groups. It brings out well the concentration of animal work in Group III laboratories, and comparison with Table 3 shows that mice are mainly responsible for this. There is a complementary dispersal of animal use

Table 4

Average number of animals used per laboratory in each group of laboratories.

	Grp. I	Grp. II	Grp. III
Number of laboratories in group (from Table 1)	182	147	26
Number of animals used (from Table 3)	350,730	769,486	621,458
Average number of animals per laboratory	1,927	5,235	23,902

in Group I, the (mostly small) diagnostic laboratories where the majority of guinea pigs are used. Dogs, cats and chickens are mostly used in Group II laboratories; although the tables do not show it, the use of dogs and cats is dispersed over many, but that of chickens concentrated in comparatively few laboratories.

Another indication of the extent to which the use of mice, guinea pigs, rabbits and rats is concentrated in a few laboratories is given in Table 5. Mice show the greatest degree of concentration. For example, 90 per cent of them,

Table 5

This table shows how far the use of mice, guinea pigs, rabbits and rats is concentrated in a smaller or greater number of laboratories.

	Mice	Guinea pigs	Rabbits	Rats
(a) No. of laboratories using each species	177+	270+	204+	116+
(b) No. of animals used in these laboratories	1,180,840	199,575	33,168	249,938
(c) No. of laboratories using, in aggregate, different percentages of (b)	(figures in brackets show percentage of (a))			
50% of (b)	5(2.8)	17(6.3)	12(5.9)	6(5.2)
60% " "	7(4)	25(9.2)	18(8.8)	8(6.9)
70% " "	9(5)	42(15.5)	27(13)	10(8.6)
80% " "	13(7.3)	56(21)	40(20)	15(13)
90% " "	27(15)	91(34)	70(34)	23(20)
95% " "	48(27)	123(45)	100(49)	40(35)

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or well over a million, are used in only 27 laboratories, an average of over 40,000 mice per laboratory. Again, 50 per cent of mice, namely 600,000, are used in only five laboratories, an average for these five of 120,000 mice per laboratory. Rabbits and guinea pigs show the least degree of concentration in use, while rats are intermediate.

It is of interest to add that the production of these species shows a similar variation in the degree of concentration, mice being produced in large numbers in a few places, while guinea pig and rabbit production is very scattered.

Comparison of some of the figures obtained from the questionnaire with figures given in the Home Office Return of Experiments (Return, 1953) for the same period is shown in Table 6. The Home Office figures include fish, but exclude animals used in laboratories but not in experiments under the Cruelty to Animals Act of 1876. The former are excluded from, but the latter are included in, the questionnaire figures. It is seen that the questionnaire accounts for about eighty per cent of the Home Office total, and this is compatible with Table 1.

Table 6

Comparison of questionnaire figures with Home Office figures for the same period.

	Home Office Figures	Figures from Questionnaires	Remarks
USES:-			
Diagnosis	202,453	217,942	Close correspondence.
Cancer research	95,991	40,065	Great error in classification under this heading.
Applied pharmacology and bioassay	over a million	972,001	Close correspondence.
SPECIES USED:-			
Cats	7,444	5,453	74%: only slightly lower than the average for all animals.
Dogs	2,830	7,510	Home Office figure does not include dogs used for serum production.
Horses	139	53	Many laboratories probably thought horses were outside the scope of the questionnaire.
TOTAL ANIMALS	2,117,912	1,741,674	81%: a good return.

Table 7 shows the number of animals of different species used for various purposes, and should be compared with Table 3.

Some explanation of the terms referring to uses may be of help here.

(a) *Diagnosis* includes all animals used in clinical pathology, both medical and veterinary, in public health work and also for pregnancy diagnosis.

Table 7

The uses of various types of laboratory animals.

	(a) Diagnosis	(b) Research	(c) Cancer Research	(d) Teaching	(e) Applied Pharmacology	(f) Bioassay	Total
Guinea pigs	117,010	38,090	44	9,388	33,811	1,232	199,575
Hamsters	60	1,322	320	10	2,934	-	4,646
Mice	90,195	288,538	15,049	11,243	669,895	105,920	1,180,840
Rabbits	8,546	16,369	275	730	6,276	972	33,168
Rats	862	94,234	21,940	6,867	75,596	50,439	249,938
Cats	-	4,250	-	609	594	-	5,453
Dogs	-	1,405	12	157	5,936	-	7,510
Ferrets	6	402	-	4	2,853	-	3,265
Cows	-	156	-	3	11	-	170
Goats	-	112	-	19	-	-	131
Horses	12	24	-	17	-	-	53
Pigs	-	24	-	-	-	-	24
Sheep	1	168	-	-	-	-	169
Primates	-	902	40	3	56	-	1,001
Other mammals	6	1,084	135	3	-	-	1,228
Canaries	-	108	-	-	-	-	108
Chickens	10	25,775	2,250	368	9,598	4,716	42,717
Pigeons	5	906	-	230	-	464	1,605
Reptiles	-	30	-	52	-	-	82
Amphibia	1,229	3,430	-	4,634	574	124	9,991
TOTAL	217,942	477,329	40,065	34,337	808,134	163,867	1,741,674

(b) *Research* is a comprehensive title, and might include such procedures as the screening of new compounds, if that is how the respondent chose to describe such work. It excludes cancer research.

(c) It was thought of interest to show *cancer research* separately.

(d) *Teaching* is self-explanatory.

(e) *Applied pharmacology* includes toxicity tests, the screening of new compounds, and the titration of sera and prophylactics; but the latter may have been shown by the respondent under the heading of bioassay, and screening as research. There is therefore no very sharp line limiting this group.

(f) *Bioassay* is self-explanatory, except that it merges into applied pharmacology, of which it is really a part. It includes vitamin D assay.

Broadly speaking, one would expect (a) to correspond closely with Group I laboratories, and indeed it does so far as guinea pigs and rabbits are concerned, but there is a considerable discrepancy in regard to mice. (b), (c) and (d) should correspond with Group II laboratories, and they do rather roughly. (e) and (f) are clearly the province of Group III laboratories, and the most striking correspondence is in the number of rats used. The reason for the discrepancies

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is that to classify a laboratory as, for example, diagnostic, does not mean that the only work done there is concerned with the diagnosis of disease. It is, of course, not necessarily so, and the discrepancies between Table 7 and Table 3 are a measure of this overlap of function.

In Table 8 the numbers and percentages of mice, guinea pigs, rabbits and rats obtained from various sources by the three groups of laboratories are shown.

Table 8

Numbers of mice, guinea pigs, rabbits and rats used in different groups of laboratories, and the sources from which they are derived. Figures in brackets are percentages of the total.

	Accredited Breeders	Dealers	Another Laboratory	Own Breeding	Total
Mice					
Group I	48,115 (4.1)	29,974 (2.5)	7,357 (0.6)	104,957 (8.9)	190,403 (16.1)
Group II	239,764 (20.3)	87,136 (7.4)	38,172 (3.2)	149,673 (12.7)	514,745 (43.6)
Group III	366,967 (31.1)	18,094 (1.5)	5,994 (0.5)	84,637 (7.2)	475,692 (40.3)
Total	654,846 (55.5)	135,204 (11.4)	51,523 (4.3)	339,267 (28.8)	1,180,840 (100)
Guinea pigs					
Group I	39,289 (19.7)	57,069 (28.6)	6,628 (3.3)	29,072 (14.5)	132,058 (66.1)
Group II	3,049 (1.5)	23,780 (11.9)	9,113 (4.6)	24,984 (12.6)	60,926 (30.6)
Group III	3,102 (1.6)	998 (0.5)	— —	2,491 (1.2)	6,591 (3.3)
Total	45,440 (22.8)	81,847 (41.0)	15,741 (7.9)	56,547 (28.3)	199,575 (100)
Rabbits					
Group I	123 (0.4)	5,394 (16.3)	106 (0.3)	4,705 (14.2)	10,328 (31.2)
Group II	580 (1.8)	12,465 (37.5)	160 (0.5)	6,639 (20.0)	19,844 (59.8)
Group III	22 -	2,576 (7.8)	12 -	386 (1.2)	2,996 (9.0)
Total	725 (2.2)	20,435 (61.6)	278 (0.8)	11,730 (35.4)	33,168 (100)
Rats					
Group I		812 (0.3)	17 -	13,926 (5.6)	14,755 (5.9)
Group II		13,588 (5.4)	8,753 (3.5)	87,765 (35.1)	110,106 (44.0)
Group III		8,534 (3.4)	118 -	116,425 (46.7)	125,077 (50.1)
Total		22,934 (9.1)	8,888 (3.5)	218,116 (87.4)	249,938 (100)

Table 9 shows a corresponding breakdown of sources of the same four species, and also of chickens, against uses.

Mice, guinea pigs and rabbits came from breeders accredited by the Laboratory Animals Bureau (Lane-Petter, 1953*b*), from other commercial sources, described for convenience as dealers, from another laboratory breeding its own animals, and a number were bred in the laboratories using them. Rats are not included in the accreditation scheme, and the comparatively small numbers purchased (22,934, or about a tenth of those bred in laboratories) is the main reason why they are not; all commercial sources of rats are therefore taken under the dealer heading.

There is a fortuitous closeness in the percentages of the three accredited species obtained from commercial sources, namely 66.9 per cent of mice, 63.8 per cent of guinea pigs, and 63.8 per cent of rabbits, and these high percentages are the reason why they were selected for accreditation. The contribution made by laboratories having a surplus of animals to the needs of other laboratories was in all cases small. Such surpluses are usually temporary, unless the laboratory is deliberately breeding in order to supply animals regularly; for example, a university or government field station.

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Table 9

Numbers of mice, guinea pigs, rabbits, rats and chickens used for various purposes, and the sourc from which they were derived. Figures in brackets are percentages of the total.

	Accredited Breeders	Dealers	Another Laboratory	Own Breeding	Total
Mice					
(a) Diagnosis of disease	48,608 (4.1)	32,554 (2.8)	1,863 (0.2)	7,170 (0.6)	90,195 (7.7)
(b) Research	68,964 (5.8)	2,586 (0.2)	2,720 (0.2)	214,268 (18.2)	288,538 (24.4)
(c) Cancer research	4,847 (0.4)	2,026 (0.2)	- -	8,176 (0.7)	15,049 (1.3)
(d) Teaching	8,080 (0.7)	263 -	200 -	2,700 (0.3)	11,243 (1.0)
(e) Applied pharmacology	439,647 (37.2)	92,510 (7.9)	34,746 (2.9)	102,992 (8.7)	669,895 (56.7)
(f) Bioassay	84,700 (7.2)	5,265 (0.4)	11,994 (1.0)	3,961 (0.3)	105,920 (8.9)
Total	654,846 (55.4)	135,204 (11.5)	51,523 (4.3)	339,267 (28.8)	1,180,840 (100)
Guinea pigs					
(a) Diagnosis of disease	39,917 (20.0)	58,810 (29.5)	6,607 (3.3)	11,676 (5.8)	117,010 (58.6)
(b) Research	1,248 (0.6)	3,921 (2.0)	261 (0.1)	32,660 (16.4)	38,090 (19.1)
(c) Cancer research	- -	- -	8 -	36 -	44 -
(d) Teaching	873 (0.4)	7,509 (3.8)	117 (0.1)	889 (0.4)	9,388 (4.7)
(e) Applied pharmacology	3,246 (1.6)	11,295 (5.6)	8,584 (4.3)	10,686 (5.4)	33,811 (16.9)
(f) Bioassay	156 (0.1)	312 (0.2)	164 (0.1)	600 (0.3)	1,232 (0.7)
Total	45,440 (22.7)	81,847 (41.1)	15,741 (7.9)	56,547 (28.3)	199,575 (100)
Rabbits					
(a) Diagnosis of disease	75 (0.2)	7,180 (21.6)	135 (0.4)	1,156 (3.5)	8,546 (25.7)
(b) Research	116 (0.4)	6,041 (18.2)	118 (0.4)	10,094 (30.5)	16,369 (49.5)
(c) Cancer research	- -	164 (0.5)	7 -	104 (0.3)	275 (0.8)
(d) Teaching	12 -	676 (2.1)	- -	42 (0.1)	730 (2.2)
(e) Applied pharmacology	522 (1.6)	5,402 (16.3)	18 -	334 (1.0)	6,276 (18.9)
(f) Bioassay	- -	972 (2.9)	- -	- -	972 (2.9)
Total	725 (2.2)	20,435 (61.6)	278 (0.8)	11,730 (35.4)	33,168 (100)
Rats					
(a) Diagnosis of disease		512 (0.2)	- -	350 (0.1)	862 (0.3)
(b) Research		3,680 (1.5)	6,402 (2.6)	84,252 (33.6)	94,234 (37.7)
(c) Cancer research		2,500 (1.0)	400 (0.2)	19,040 (7.6)	21,940 (8.8)
(d) Teaching		2,330 (0.9)	48 -	4,489 (1.9)	6,867 (2.8)
(e) Applied pharmacology		6,920 (2.8)	538 (0.2)	68,138 (27.2)	75,596 (30.2)
(f) Bioassay		6,992 (2.8)	1,500 (0.6)	41,947 (16.8)	50,439 (20.2)
Total		22,934 (9.2)	8,888 (3.6)	218,116 (87.2)	249,938 (100)
Chickens					
(a) Diagnosis of disease		4 -	6 -	- -	10 -
(b) Research		24,087 (56.4)	1,536 (3.5)	152 (0.4)	25,775 (60.3)
(c) Cancer research		- -	2,000 (4.7)	250 (0.5)	2,250 (5.2)
(d) Teaching		368 (0.9)	- -	- -	368 (0.9)
(e) Applied pharmacology		5,898 (13.8)	3,700 (8.7)	- -	9,598 (22.5)
(f) Bioassay		2,716 (6.4)	8,000 (7)	2,000 (4.7)	4,716 (11.1)
Total		33,073 (77.5)	7,242 (16.9)	2,402 (5.6)	42,717 (100)

Question 2

Question 2 asked for reasons why any particular source of animals was chosen. The object was to see to what extent consistent policy dictated the choice of sources. For simplicity, all sources have been grouped in two alternatives, "own breeding" and "purchasing." Only mice, guinea pigs, rabbits and rats are here considered: the reason for a particular choice of source in other species is nearly always unequivocal and in any case comparatively unimportant.

Reasons for choosing "own breeding" or "purchasing" come under four headings:—

(a) Because the animals are better, i.e., more healthy, more uniform in strain, or subjected to a more carefully controlled and known environment.

(b) Because they are cheaper: but in many cases there was no indication that a proper cost comparison had been made. It has already been pointed out that most laboratories appeared very uncertain in the matter of costs.

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(c) Because of convenience: it is obviously difficult or impossible to purchase certain sorts of animal, e.g., littermates, newborns, or those with a specified prenatal dietary history. There is also the question of proximity and reliability of supply, and the absence of room for laboratory breeding or of an alternative source.

(d) Many preferences were unspecified: the laboratory was satisfied as things were, and did not wish to make any change.

Table 10 shows which preference was expressed by laboratories of the three groups, and for what reasons. The total preferences correspond well enough with

Table 10

The number of laboratories showing preference for breeding or purchasing mice, guinea pigs, rabbits and rats and giving reasons for their choice.

	Preference for breeding					Preference for purchase				
	Better	Cheaper	Convenience	Undefined	Total	Better	Cheaper	Convenience	Undefined	Total
Mice										
Group I	14	2	10	1	27	14	5	21	12	52
Group II	39	4	10	3	56	18	8	18	11	55
Group III	6	3	1	-	10	5	1	4	3	13
Total	59	9	21	4	93	37	14	43	26	120
Guinea pigs										
Group I	21	29	36	1	87	31	7	62	25	125
Group II	20	5	11	-	36	15	-	23	12	55
Group III	9	1	3	-	13	3	1	4	2	10
Total	50	35	50	1	136	49	13	89	39	190
Rabbits										
Group I	11	10	16	2	39	19	5	27	17	68
Group II	24	4	13	-	41	20	8	34	17	79
Group III	7	2	3	-	12	5	1	5	1	12
Total	42	16	32	2	92	44	14	66	35	159
Rats										
Group I	3	1	5	-	9	-	1	3	1	5
Group II	41	5	20	2	68	12	2	20	8	42
Group III	12	1	-	-	13	2	2	1	2	7
Total	56	7	25	2	90	14	5	24	11	54

the numbers used from different sources (cf. Table 8). A majority of laboratories prefers to purchase mice, guinea pigs and rabbits, and to breed rats.

Question 3

In the answers to Question 3 the relative importance of different causes of loss among laboratory animals is examined. Of 355 laboratories answering Question 3, 167 (47 per cent) reported no losses at all. The other 188 gave details of their losses, and these details are shown in Table 11, in respect of mice, guinea pigs, rabbits and rats.

Miscellaneous diseases include a number of diseases not clearly belonging to any of the other groups; none affected more than a few animals in any one outbreak.

Table 11

Causes of losses in mice, guinea pigs, rabbits and rats. Figures in brackets show percentages of total used which were lost from various causes.

	Mice	Guinea pigs	Rabbits	Rats
Total used (from Table 3)	1,180,840	199,575	33,168	249,938
Total lost	21,651 (1.84)	4,061 (2.05)	513 (1.54)	5,090 (2.05)
Causes of death:-				
1. Specific infections:-				
Salmonellosis	3,080	757	-	-
Pasteurellosis	43	348	14	3
Tyzzer's disease	8,500	-	-	-
Ectromelia	2,522	-	-	-
Coccidiosis	-	92	217	-
Total	14,145 (1.20)	1,197 (.60)	231 (.69)	3
2. Non-specific disease:-				
Respiratory	13	665	48	78
Intestinal	287	228	61	22
Ear	-	-	-	85
Miscellaneous	55	189	27	46
Total	355 (.03)	1,082 (.54)	136 (.41)	231 (.09)
3. Natural causes	6,159 (.52)	404 (.22)	146 (.44)	4,696 (1.88)
4. Undiagnosed	992 (.09)	1,378 (.69)	-	160 (.06)

Natural causes include causes of death not directly attributable to disease, such as stillbirths, neonatal deaths, bad mothering, infanticide, cannibalism and old age; trauma from accident and from fighting; inadequate accommodation, poor management and faulty nutrition; unthriftiness; congenital abnormalities; and losses in transit. Mice and, in proportion to their total number, more particularly rats are most heavily affected under this heading.

Many reported losses were undiagnosed, especially among guinea pigs.

It was not possible to correlate losses with sources, but some indication of this is given below in Table 12.

An attempt to correlate causes of death with time of the year was only partially successful. Respiratory infections occurred more frequently during the winter and spring months, intestinal during the early spring and summer, and most other infections in sporadic outbreaks at any time of the year. Losses from natural causes were more frequent in the autumn and winter.

Very little information was given about losses in animals other than the four species already mentioned.

Question 4

The opinions expressed about which source provided better animals are summarised in Table 12, which analyses the answers to Question 4; again only

mice, guinea pigs, rabbits and rats are shown. The complaints reported were of many kinds: infection; animals under weight, wrong size, or of poor quality; wrong strain or pedigree; responding abnormally or irregularly in use; too expensive; wrong sex, or pregnant; and insufficient in numbers. All these reasons for complaint could be partly or wholly blamed on the source of supply, and are included in the analysis. Many other complaints were listed which could not fairly be so attributed, and these have been ignored.

Table 12

The number of laboratories reporting complaints about the quality of the animals, and the sources of the unsatisfactory animals. The figures in brackets are the numbers of complaints per thousand animals supplied. A fraction indicates that the same laboratory obtained animals from different sources and did not specify which were unsatisfactory: the blame has then been shared equally among the sources.

	Accredited Breeder	Dealer	Another Laboratory	Own Breeding
Mice	$10\frac{1}{2}$ (0.016)	$9\frac{1}{2}$ (0.07)	nil	11 (0.032)
Guinea pigs	6 (0.13)	$28\frac{1}{6}$ (0.35)	$2\frac{1}{2}$ (0.16)	$4\frac{1}{3}$ (0.77)
Rabbits	2 (2.8)	$30\frac{1}{2}$ (1.5)	1 (3.6)	$5\frac{1}{2}$ (0.47)
Rats	-	$13\frac{1}{6}$ (0.58)	$2\frac{1}{3}$ (0.26)	$5\frac{1}{2}$ (0.025)

Examination of Table 12 shows that the accredited mouse breeder gave the greatest satisfaction, although "own breeding" was not far behind. The dealer was plainly a poor source. "Another laboratory" scored no complaints, but accounted for only 4.3 per cent of the total. Accredited guinea pig breeders showed up very well in comparison with either dealers or "own breeding": their apparent superiority should be compared with the evenly balanced opinions expressed in Table 10. On the other hand, rabbits bred in the laboratory appeared markedly better than those from other sources; and of the latter, the dealer had the advantage over the accredited breeder. Rats were, as one would expect, better bred than bought.

Question 5

The preference often expressed for the use of inbred strains led to the inclusion of Question 5. Comparatively few laboratories use inbred strains, and only mice, guinea pigs, rabbits and rats are shown in Table 13. It will be seen that rather more laboratories used inbred rats than inbred mice; the reason is largely the popularity of the strain of Wistar albino rat introduced into this country about thirty years ago and since distributed to many laboratories.

Questions 6, 7 and 8

Question 6 did not call for analysis. The information provided here led to the compilation of the *Catalogue of Uniform Strains of Laboratory Animals*

Table 13

The number of laboratories using inbred animals, and the purposes for which they use them.

	Mice	Rabbits	Guinea pigs	Rats
Diagnosis	-	1	1	-
Research (genetics)	4	-	-	-
Research (cancer)	11	-	-	5
Other research	7	1	6	12
Applied pharmacology	3	4	2	2
Bioassay	1	-	-	10
TOTAL	26	6	9	29

maintained in Great Britain (q.v.). The answers to Question 7 were of purely internal interest to the Laboratory Animals Bureau. The same could be said of Question 8; many interesting and useful comments appeared here.

Part II.—The Discussion

In recent years much attention has been paid to the provision of animals for research and other laboratory uses. Lane-Petter (1953*a*) and Paterson (1953) have discussed this question at some length, and many different suggestions have been put forward for meeting the demand. Moreover, this demand is not only growing in quantity year by year, but qualitative requirements tend to be more exacting than in the past. Laboratory animals are to-day costing more than they have ever done before.

Cost, however, is only one side of the problem, and unfortunately one on which there is the least accurate information available. In any scheme for the provision of animals, it is necessary to arrange for maximum flexibility of supply, for demand often fluctuates to an extent that cannot be accommodated within a normal breeding programme. It is usually expensive for a laboratory to breed all its own animals, often inconvenient, and sometimes inefficient. Unless workers are from time to time to be kept waiting unduly for their material, it is also wasteful, for permanent surpluses have to be carried to meet demands. It is at this stage, therefore, that the necessity for planning the supply becomes evident, and it is desirable that this planning shall be based on as broad a foundation of fact as can be ascertained.

Are large breeding stations a rational answer to the question of supply? If so, how large should they be? Should they serve single universities, groups of universities, or local groups including a variety of users? How should the cost of such breeding stations be distributed, and can they be expected to pay their way? Is there a case for research into methods of animal production, and where

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is the cost of this to fall? That these questions are of moment is implicit in the very existence of the Laboratory Animals Bureau, and it is clearly a proper function of such a unit to seek the sort of information which the present questionnaire attempted to elicit.

This questionnaire caused a great many people a lot of trouble. One is obliged to inquire how far the above results have justified this. Was the information being sought really needed? Was it obtained? Once obtained, will it prove useful? How much reliance can be placed on the figures? Have any important or unexpected facts come to light? What conclusions can be drawn from this report?

Was the information being sought really needed?

Questionnaires of narrower scope and distribution had previously been sent out by the Laboratory Animals Bureau, and before that by the committee on the supply of experimental animals set up in 1943. The last general circulation was at the end of 1949, but one or two limited inquiries had been carried out since that date. Previous attempts to throw light on this matter before the present questionnaire was examined have been little more than informed guesswork. It is very much better to have hard facts.

Was the information obtained?

Obtaining information in this way is dependent on the design of the questionnaire, the manner in which it is distributed, and the co-operation of the respondents.

It has already been pointed out that in the design a compromise was struck between the mechanical type of questionnaire with a multiplicity of questions, and the type which invites essay-length answers. The framing of the questions was such as to avoid ambiguity of meaning, a constant pitfall in all questionnaires, and with one exception this object was achieved. Even the exception, the question of "cost per animal" (Question 1, Column E), was only half a failure, for it was realised beforehand that those laboratories that knew their cost figures used different methods of arriving at them, and therefore a definition would hardly have helped. But it transpired that costing is too seldom done on any basis, more especially when laboratories breed their own animals.

The distribution was virtually complete for all laboratories in the country using animals, with the exception of the one or two cases where blocks of questionnaires were sent for local distribution which was not carried out. The co-operation of respondents was good.

Will the information prove useful?

The answer to this depends on how far experience in 1952 can be used in subsequent years. Unless there are some quite fundamental changes in the methods of production, procurement and use of any animals, the 1952 figures

will serve as a guide for many years to come. Allowing for the fact that every laboratory, large or small, has its own parochial problems in this field, no plan of animal provision should ignore the foregoing results.

How much reliance can be placed on the figures?

Table 1 shows that one quarter of the questionnaires were not received back, but in fact a somewhat smaller proportion of laboratories using animals failed to supply information. It may be taken as a working basis for calculation that the return represents about an 80 per cent sample, and that this sample is reasonably representative. Very few large-scale users failed to provide information.

There is nothing to suggest that the figures actually given were anything but entirely accurate. In a few cases the appearance of round numbers might mean that they were near approximations, but if this is so it is unlikely that any significant error was introduced. It is considered that the figures are more than sufficiently accurate for any conclusions that may be drawn from them.

Have any important or unexpected facts come to light?

A study of the numbers of different species of animal, the laboratories where they were used, and the uses they were put to, gives an indication of the relative economic importance of each species. The great preponderance of five species, and among these the greater preponderence of mice, is striking. Emphasis is placed on their economic importance advisedly. The cost of animals used in small numbers is insignificant in relation to that of the work for which they are used, but when they come to be used in large numbers the question of getting value for money is important. It is also important in the case of the more expensive animals, particularly cats, dogs and monkeys.

It is, therefore, perhaps surprising that so little information was provided about the cost of animals, and that the answers to Question 2, as shown in Table 10, were unconvincing. The inference is that care given to the choice of source and to the economical provision of animals is likely to lead to appreciable saving of money and effort, and may at the same time serve the user to better advantage.

Conclusions

While it is recognised that every department and institute has its own special requirements and resources, it is nevertheless true that the facts and figures brought to light in this survey are likely to have some relevance in almost all laboratories that are interested in providing the most suitable animals at the most economic price.

No rule can be laid down whether to buy or breed, how to group resources, or to what extent special requirements such as genetic purity or freedom from infection are necessary; these are all matters for local consideration. But local considerations should show good reasons for departing from the general principles emerging from this survey.

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Procurement policies must be planned. The choice of sources of animals should depend on facts, rather than on custom, convenience or uninformed preference. There must, of course, never be any denial of the need for each worker to have the animal best suited to his work. It would perhaps help him if he knew what choice of sources was open to him, and how much his animals were costing his department. He should be able to seek advice, preferably on the spot, about this, and the obvious person to consult is the person in charge of the animal department—call him curator, superintendent, or what you will, so long as he is a member of the senior staff, and can carry both authority and respect.

This implies that the curator should have a reasonable realm of interest, which in turn points to the advantage of some degree of grouping of laboratories, on a university, regional or service basis. Planning of supply is desirable; it is susceptible to informed supervision; it is more efficient if there is sufficient grouping of consumer interests to make it worth the trouble of at least a part-time curator; and it is likely to be economic. To-day much of the information relevant to this is available, curators do exist and are justifying their existence, and all the time more knowledge is being acquired in this field.

The survey reveals that a large proportion of laboratories had little or no knowledge of the cost of their animals. This is regrettable, and suggests that some attempt at costing this side of expenditure should be made. More deliberate organisation of animal supplies would necessarily involve better costing, and indicate what is the true cost of animals, as opposed to the net cost of breeding or purchase. Cheap animals may prove expensive and frustrating in the long run but paying more does not automatically result in getting better animals.

It is difficult to understand why over half the laboratories reported no losses during the year, while the other 47 per cent showed losses among mice, guinea pigs, rabbits and rats of approximately 2 per cent of the total of each species used. A likely explanation is that those laboratories reporting no losses had no records of any losses sustained, and it is probably safe to multiply the figures and percentages in Table 11 by a factor of 2 or more.

The distribution of losses is informative. Specific infection, notably Tyzzer's disease, was by far the most important killer of mice, with natural causes a bad second. Natural causes accounted for nearly all the losses of rats, a fair number of rabbits, but very few guinea pigs. Non-specific infection affected mostly the guinea pigs and rabbits, while over a third of the guinea pig losses were undiagnosed.

The conclusions are clear. There is need for improvement of the standard of hygiene and control of infection in mice in particular, and also in rabbits and guinea pigs. The general standard of husbandry and nutrition of rats is not what it might be, and the same applies to a less extent with mice and rabbits. Intestinal and respiratory disease cause serious wastage among rabbits and guinea pigs, especially in the spring; again hygiene and also ventilation may be at fault. Lastly, causes of death should always be recorded and wherever possible diagnosed.

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The use of inbred strains is not perhaps as widespread as might have been thought. It is wrong to assume that inbred animals will automatically give better results for every purpose; but it is equally wrong to fail to consider the possibility of their use.

Summary

1. A questionnaire was sent to all laboratories using animals in Great Britain, asking for information about their experiences during 1952.

2. Responses estimated at 80 per cent were received.

3. Most of the information contained in the replies is given in tabular form.

4. This information is of interest to all who have the responsibility of providing animals for laboratory use.

5. It appears that at present the sources of animals chosen by laboratories are not always those that serve their interests best.

6. The provision of animals is capable of rational planning, for the sake of economy and of suiting the animal to the need.

7. A pointer is given to the directions in which there is most need for improvement in the management of different species.

Acknowledgment

Our thanks are due to all those who responded to the request for information, and who completed with care a tedious questionnaire. In fairness to them we have tried to extract the maximum amount of useful information.

REFERENCES

Catalogue of Uniform Strains of Laboratory Animals Maintained in Great Britain, published by the Laboratory Animals Bureau.
Lane-Petter, W. (1953*a*): *Laboratory Animals Bureau Collected Papers, 1,* 7.
Lane-Petter, W. (1953*b*): *Mon. Bull. Min. Hlth., Publ. Hlth. Lab. Serv., 12,* 165.
Paterson, J. S. (1953): *Laboratory Animals Bureau Collected Papers, 1,* 55.
Return (1953): *Return of experiments performed under the Cruelty to Animals Act, 1876, during 1952,* H.M.S.O.

SPECIAL NUMBER

THE September number of the JOURNAL will be enlarged and have special reference to the British Veterinary Association Congress Meeting, which this year is planned to take place in Belfast from September 4 to September 10.

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OBSERVATIONS ON THE UREA CONTENT OF THE BLOOD OF CALVES AND ADULT CATTLE

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THERE is little information available on the blood urea levels of normal calves and adult cattle and tests have therefore been made in order to ascertain the average blood urea content of these animals over a twelve-month period, and the variations which may occur between different breeds and ages, and between pregnant and non-pregnant animals. Consideration was also given to the possibility that the milk yield may have an effect on the amount of urea in the blood of lactating animals.

As the management and diet of the animals influences the production of urea, estimations were made on calves and adult cattle that were at grass and received calf nuts, cake, mangolds or hay as additional ingredients to their ration. Two of the herds were housed during January and February on two occasions because of the weather.

The study of the blood of the calves was made according to age and four recordings were made each week from birth to one year of age. This gave 212 readings and 80 calves had therefore to be kept under observation. The study of the blood of adult cattle was made by selecting four adult animals from each of four herds, namely, Guernsey, Shorthorn, Friesian and Ayrshire: these were bled at monthly intervals for a period of one year, thus giving 192 recordings.

Technique

All samples of blood were taken from the jugular vein of apparently healthy animals in attested herds; potassium oxalate at the rate of 1 mg. per ml. of blood was used as the anticoagulent. An M.R.C. Grey-Wedge Photometer was used for the determination of the urea content and the method adopted was that described for use with this particular instrument. B.D.H. urease tabloids were used for the urease suspensions which were used within 48 hours of preparation.

Results

(*a*) CALVES

All the calves were kept as near as possible under the same environmental conditions, most being at pasture. During the 52-week period the range of blood urea was between 28.0 and 134.0 mg. per 100 ml. of blood, with means varying between 43.2 and 90.7 mg. per 100 ml. of blood. The values recorded during the first three months of life are higher than those obtained from calves between the age of three to six months. From the age of six months there is a gradual increase which progresses to the end of the period under investigation. These are depicted in Tables I and II, and Fig. 1.

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TABLE I THE BLOOD UREA CONTENT OF CALVES FROM BIRTH TO 52 WEEKS OF AGE (MG PER 100 ML BLOOD).

AGE IN WEEKS	BIRTH.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
CALF NO 1	85·0	62·4	90·0	48·0	70·8	78·4	41·8	78·4	55·4	70·2	101·4	67·4	55·4	44·4	55·4	62·8	70·2	32·0	45·8	62·8	45·0	70·2	64·8	66·6	72·2	85·0	68·4
NO 2	88·0	92·0	90·2	84·0	50·0	55·4	59·2	74·0	66·6	66·6	40·6	62·8	74·0	59·2	70·2	62·8	66·6	55·0	29·0	56·6	45·0	45·0	43·7	55·4	70·8	77·6	59·2
NO 3	59·2	87·0	52·5	91·7	104·0	79·6	59·2	85·0	51·8	70·2	74·0	75·0	66·6	88·8	99·9	67·2	44·6	55·0	32·0	82·6	60·2	43·4	57·5	64·1	48·0	28·0	66·6
NO 4	77·6	39·8	55·0	43·6	90·6	67·5	49·1	58·4	53·2	54·7	55·3	70·2	48·0	66·6	34·0	63·6	43·3	58·3	66·1	78·0	98·8	50·4	47·1	31·9	61·8	43·3	60·8
MAX	85·0	92·0	90·2	91·7	104·0	79·6	59·2	85·0	66·6	70·2	101·4	75·0	74·0	88·8	99·9	67·2	70·2	58·3	66·1	82·6	98·8	70·2	64·8	66·6	72·2	85·0	68·4
MIN	59·2	39·8	52·5	43·6	50·0	55·4	41·8	58·4	51·8	54·7	40·6	62·8	48·0	44·4	34·0	62·8	43·3	32·0	29·0	56·6	45·0	43·4	43·7	31·9	48·0	28·0	59·2
MEAN	76·7	70·3	71·9	66·8	78·8	70·2	52·3	73·9	56·7	65·1	67·8	68·8	61·0	67·2	64·8	64·1	56·1	50·0	43·2	70·0	62·2	52·2	53·2	54·5	63·2	58·4	63·7
S.D.	10·5	20·9	18·2	21·4	20·4	9·8	7·3	9·8	5·8	6·4	22·7	4·4	10·0	16·2	23·9	1·8	12·3	10·5	14·8	10·7	22·0	10·7	8·4	13·7	9·7	23·6	3·8
C. OF V.	13·7	29·7	25·3	32·0	25·9	13·9	13·9	13·3	10·2	9·8	33·5	6·4	16·4	24·1	36·9	2·8	21·9	21·0	34·3	15·3	35·4	20·5	15·8	25·1	15·3	40·4	5·9

AGE IN WEEKS	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	MIN.	MAX	MEAN
CALF NO 1	41·8	47·4	48·1	124·2	61·6	74·0	55·4	50·0	92·9	59·2	62·8	81·4	85·0	55·4	51·8	77·6	89·6	70·2	59·2	48·0	74·0	85·0	74·0	66·6	55·8	79·6	124·2	32·0	66·4
NO 2	52·5	78·1	89·6	80·0	120·0	81·4	55·4	77·6	74·0	70·2	74·0	83·2	74·0	70·2	62·8	56·4	52·6	73·4	96·6	90·0	58·0	101·8	89·8	101·8	53·6	83·8	120·0	29·0	69·8
NO 3	83·6	67·4	100·6	68·0	70·0	69·2	77·6	59·2	74·0	88·8	71·2	70·2	62·8	77·6	55·4	93·2	134·0	83·8	109·8	79·0	62·8	81·4	74·0	96·2	93·2	51·8	134·0	28·0	72·6
NO 4	90·0	110·6	72·4	88·8	75·0	95·2	89·1	52·5	95·6	73·2	66·6	66·6	72·6	105·8	75·4	48·6	86·7	80·3	86·6	85·8	84·0	78·2	73·0	51·8	81·4	89·7	110·6	31·9	68·6
MAX	90·0	110·6	100·6	124·2	120·0	95·2	89·1	77·6	95·6	88·8	74·0	83·2	85·0	105·8	75·4	93·2	134·0	83·8	109·8	90·0	84·0	101·8	88·8	101·8	93·2	89·7			
MIN	41·8	47·4	48·1	68·0	61·6	69·2	55·4	50·0	74·0	59·2	62·8	66·6	62·8	55·4	51·8	48·6	52·6	70·2	59·2	48·0	58·0	70·2	73·0	51·8	53·6	51·8			
MEAN	66·9	75·8	77·6	90·2	81·6	84·9	69·8	59·8	84·1	72·8	65·6	75·3	73·6	77·2	61·3	63·9	90·7	76·9	88·0	75·7	72·2	84·6	77·4	79·1	71·0	77·4			
S.D.	20·8	22·9	19·8	20·9	22·7	11·0	14·5	10·8	10·2	10·5	4·3	7·1	7·8	18·3	9·0	18·3	28·9	5·4	18·6	17·2	10·4	11·4	6·6	20·7	16·5	15·3			
C. OF V.	30·3	30·2	25·5	23·2	27·8	12·9	20·9	18·1	12·1	14·4	6·3	9·4	10·6	23·7	14·7	28·6	31·9	7·0	21·1	22·7	14·4	13·5	8·5	26·2	23·7	19·8			

S.D. = STANDARD DEVIATION ± C. OF V. COEFFICIENT OF VARIATION.

TABLE 2.

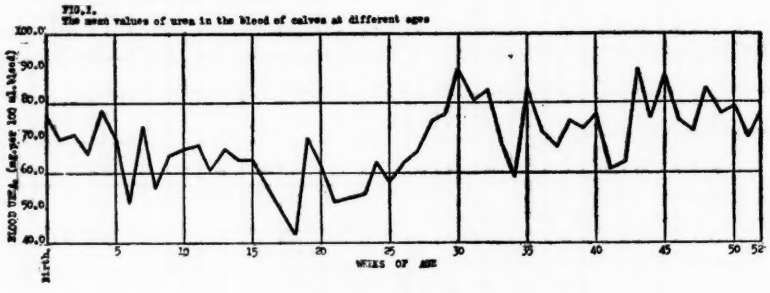
Urea Content of Blood of Calves according to Age

AGE	Recorded Weekly figures (mg. per 100 ml of blood)		Mean Ranges (mg. per 100 ml of blood)	
	Min.	Max.	Min.	Max.
Birth to 13 weeks	39.8	104.0	52.3	78.8
14 weeks to 26 weeks	28.0	99.9	43.2	70.0
27 weeks to 39 weeks	41.8	124.2	59.8	90.2
40 weeks to 52 weeks	48.0	134.0	61.3	90.7

There is apparently no significant difference between the results from calves at pasture and those of the same age kept under housed conditions. When the mean values for the months of the year are considered, however, higher figures were obtained between July and October than during the other months of the year.

Significant differences in the data were found for animals of the same age and the mean values were plotted in a curve as shown in Fig. 1. These differences for the calves of different ages were shown on an analysis of variance to have a probability of <0.05 to >0.01.

FIG. 1.
The mean values of urea in the blood of calves at different ages



(*b*) Adult Cattle

Four cows of different ages were selected at random from attested herds of four different dairy breeds, namely, Guernseys, Shorthorns, Friesians and Ayrshires.

The herds chosen were kept under identical conditions of management and on the same type of diet. The Guernsey and Shorthorn herds were housed for short periods during December, January and February on account of weather conditions, but the Friesian and Ayrshire herds were at pasture throughout the year.

Guernseys.

The blood urea content of the four Guernsey cows ranged from 26.0 to 128.4 mg. per 100 ml. of blood, with a mean value of 64.7 mg., which was lower than that obtained with the other three breeds. The mean value for each individual cow was 63.9, 65.6, 59.8 and 69.7 mg.

XUM

Shorthorns.

The range recorded for the four shorthorn cows was from 32.0 to 167.2 mg. per 100 ml. of blood, the mean being 78.1 mg. The mean values for the individual cows were slightly higher than those recorded in the Guernsey cattle, namely, 75.6, 77.3, 82.0 and 77.8 mg.

Friesians.

From the figures recorded there is little difference between the blood urea content of Friesians, Shorthorns and Ayrshire cows. The range in the Friesian herd was from 29.4 to 142.2 mg. per 100 ml. of blood with a mean of 76.5 mg.; mean figures for the four cows were 88.5, 72.0, 70.2 and 75.4 mg.

Ayrshires.

The range for this herd was from 25.3 to 167.2 mg. per 100 ml. of blood with a mean value of 76.4 mg.; mean values for the four cows were 76.4, 80.5, 73.9 and 74.9 mg.

From Tables IV, V and VI it is seen that high recordings were obtained in October in the cows of the Shorthorn herd, in April with the Friesians and November in the Ayrshires. Repeated tests were made on all such samples to eliminate the possibility of technical error and there were no reported changes of management.

The figures recorded for the four herds showed a variance ratio of 3.81 with a significance of 0.01 in the difference *between* breeds, and a variance ratio of 1 in the difference *within* breeds.

TABLE 3. BLOOD UREA CONTENT OF FOUR GUERNSEY COWS.

ANIMAL	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	MAX	MIN	MEAN	S.D.	C. of V.
No 1	104·0	114·3	26·0	57·0	49·2	76·9	52·8	75·4	71·4	69·0	76·0	64·0	104·0	26·0	63·9	19·1	29·9
No 2	68·0	46·5	31·4	24·3	35·6	121·4	47·4	73·0	82·1	76·6	80·0	89·0	121·4	24·3	65·6	28·1	42·8
No 3	58·0	38·6	39·8	22·7	40·8	74·0	60·2	72·1	75·0	64·0	82·0	80·0	82·0	22·7	59·8	17·7	29·6
No 4	62·0	51·3	49·1	43·8	52·1	90·8	66·6	70·8	73·4	76·2	92·2	104·0	104·0	43·8	69·7	18·4	26·4
MAX.	104·0	51·3	49·1	57·0	52·1	121·4	66·6	77·0	82·1	76·6	92·2	104·0					
MIN.	58·0	38·6	26·0	24·3	35·6	74·0	47·4	70·8	71·4	64·0	76·0	64·0					
MEAN	73·0	48·1	36·5	38·9	44·4	92·5	57·0	73·8	76·7	72·7	82·5	84·0					
S.D.	18·3	4·6	9·8	12·4	6·1	21·6	7·2	1·5	3·9	5·1	5·9	4·6					
C. of V.	25·1	10·2	24·1	32·0	15·0	23·3	12·6	2·4	5·1	5·1	7·1	5·7					

S.D. = STANDARD DEVIATION ±

C. of V. = COEFFICIENT OF VARIATION

TABLE 4 BLOOD UREA CONTENT OF FOUR SHORTHORN COWS.
(MG PER 100 ML BLOOD)

ANIMAL	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	MAX	MIN	MEAN	S.D.	C.OF V.
No 1	64·0	90·0	60·0	53·2	81·4	74·0	98·8	101·8	93·2	140·0	64·3	74·0	140·0	53·2	82·0	22·5	27·4
No 2	76·0	62·0	68·0	73·2	74·0	51·8	81·4	88·8	64·8	160·0	63·4	72·4	160·0	51·8	77·8	26·5	34·1
No 3	68·0	62·0	50·0	60·0	81·4	51·8	85·0	93·2	83·2	167·2	60·6	66·4	167·2	50·0	77·3	30·1	38·9
No 4	72·0	58·0	32·0	63·2	55·4	44·4	81·4	81·4	93·2	162·0	71·0	94·0	162·0	32·0	75·6	30·7	40·6
MAX	76·0	90·0	68·0	73·2	81·4	74·0	98·8	101·8	93·2	167·2	71·0	94·0					
MIN	64·0	58·0	32·0	53·2	55·4	44·4	81·4	81·4	64·8	140·0	60·6	66·4					
MEAN	70·0	68·0	52·5	62·4	73·0	55·5	84·1	91·3	83·6	157·8	64·5	76·7					
S.D.	4·9	12·8	13·8	7·2	10·6	10·9	3·0	7·5	11·6	10·3	4·0	10·3					
C.OF V.	6·9	18·8	25·3	11·3	14·5	19·6	3·6	8·2	13·9	6·5	6·2	13·4					

S.D. STANDARD DEVIATION ±
C.OF V. = COEFFICIENT OF VARIATION.

TABLE 5. BLOOD UREA CONTENT OF FOUR FRIESIAN COWS
(MG PER 100 ML BLOOD)

ANIMAL	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	MAX	MIN	MEAN	S.D.	C.OF V.
No 1	96·0	54·9	60·7	81·5	43·8	56·0	64·0	98·2	79·2	77·6	90·4	72·0	98·2	43·8	72·0	17·3	24·0
No 2	72·0	59·8	61·2	107·4	43·8	93·8	76·8	83·6	125·6	142·2	118·2	90·0	142·2	43·8	88·5	28·1	31·8
No 3	80·0	57·3	66·7	109·4	45·8	62·8	28·4	63·8	61·3	82·4	103·4	84·0	109·4	28·4	70·2	21·9	31·2
No 4	60·0	83·6	74·2	105·3	39·2	90·8	30·2	93·2	69·2	98·8	84·4	90·0	105·3	30·2	75·4	21·7	28·8
MAX	96·0	83·6	74·2	109·4	45·8	93·8	76·8	93·2	125·6	142·2	118·2	84·0					
MIN	60·0	54·9	60·7	81·5	39·2	56·0	28·4	63·8	61·3	77·6	84·4	72·0					
MEAN	77·0	63·9	66·2	100·9	43·1	75·9	47·6	85·9	83·3	97·7	99·3	79·5					
S.D.	13·1	11·5	5·8	11·3	2·4	16·4	19·6	14·1	23·2	26·1	13·4	24·9					
C.OF V.	17·0	17·9	9·0	11·2	5·6	21·9	41·2	16·4	27·9	26·7	13·5	31·3.					

S.D. = STANDARD DEVIATION ±
C.OF V. = COEFFICIENT OF VARIATION.

TABLE 6 BLOOD UREA CONTENT OF FOUR AYRSHIRE COWS.
(MG PER 100 ML BLOOD)

ANIMAL	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	MAX	MIN	MEAN	S.D.	C.OF V.
No 1	106·0	80·0	76·0	78·0	78·0	30·0	30·3	90·2	77·4	43·6	110·0	87·4	110·0	30·0	73·9	23·6	31·9
No 2	68·0	85·0	82·5	71·2	74·5	47·0	50·2	100·0	93·6	47·6	106·0	96·0	106·0	47·0	80·5	19·8	24·6
No 3	88·0	93·3	87·6	90·0	86·9	45·0	45·7	123·0	72·4	39·4	76·0	80·0	123·0	39·4	76·4	22·5	29·5
No 4	79·0	80·0	70·6	72·8	71·5	100·0	25·3	94·0	62·6	54·1	100·0	90·0	100·0	25·3	78·9	20·3	27·1
MAX	106·0	85·0	87·6	90·0	86·9	100·0	50·2	123·0	93·6	54·1	110·0	95·0					
MIN	78·0	80·0	70·6	71·2	71·5	30·0	25·3	90·2	62·6	39·4	76·0	80·0					
MEAN	95·0	93·0	79·1	78·0	78·9	56·5	37·3	101·8	76·5	46·0	98·0	88·1					
S.D.	17·3	3·2	6·4	7·4	5·4	26·5	10·4	12·8	11·3	5·5	13·2	5·4					
C.OF V.	18·2	3·7	8·1	9·5	6·8	47·7	27·5	13·6	14·8	11·9	13·5	6·1					

S.D. = STANDARD DEVIATION ±
C.OF V. = COEFFICIENT OF VARIATION.

XUM

Variation With Age.

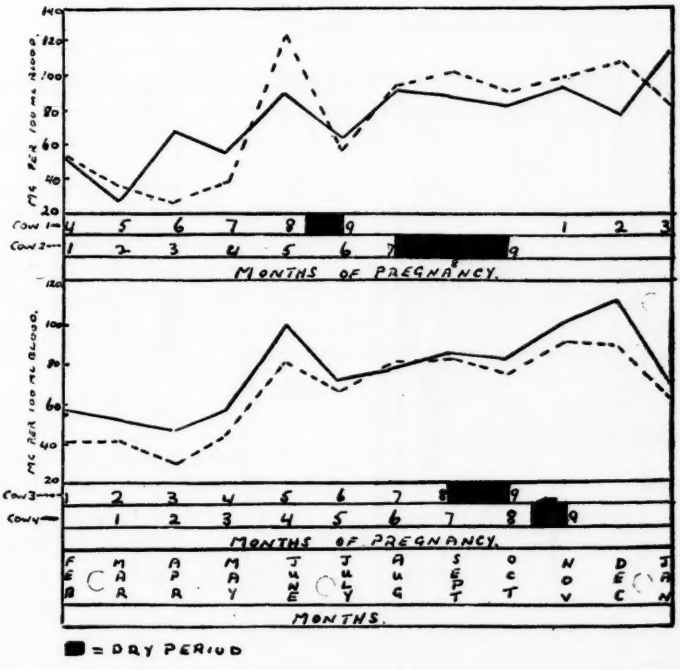
The ages of the cows in the herds ranged from 3 to 6 years, and the mean figures show a slight tendency to increase with the age of the animal.

Age	*mg. of urea per 100 ml. of blood*
3 years	70.0
4 „	74.8
5 „	75.0
6 „	75.6

Variation With Season.

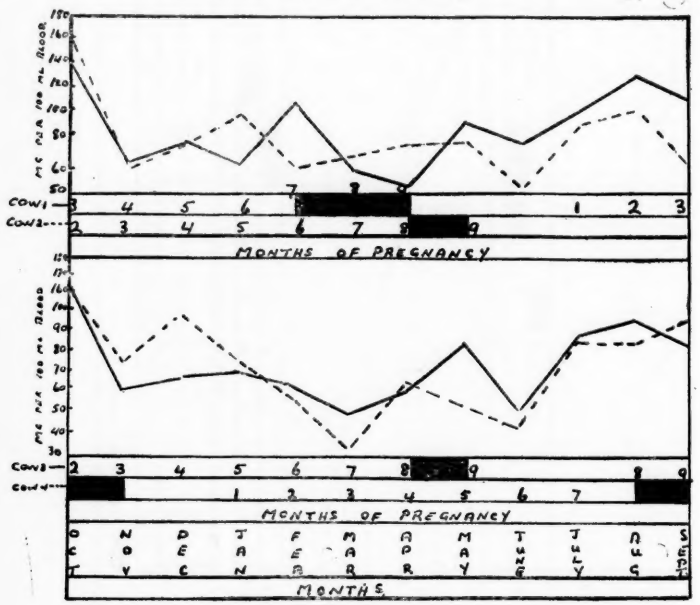
Except for the occasions when it became necessary to house the Guernseys and Shorthorns, all the animals were exposed to the same seasonal conditions. Higher figures were frequently recorded when the animals were bled on warm days in summer and winter, whilst on cold days, lower values were obtained. When the *monthly* figures for the four herds are taken and worked out on a statistical basis, it is found that the variance ratio for the months of the year is 4.6 with a significance of <0.001. Rises in the blood urea content of all the cows were noticeable during the month of August; no satisfactory explanation could be found for this, except that the weather was fine and warm on each occasion the animals were bled. Mean figures for quarterly periods

FIG 2. VARIATIONS OF BLOOD UREA OF FOUR GUERNSEY COWS DURING PREGNANCY.



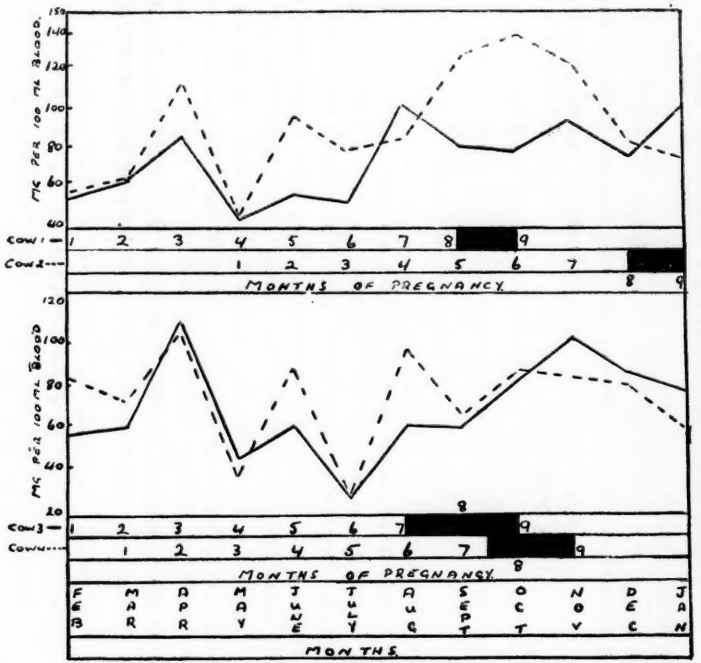
■ = DRY PERIOD

FIG 3. VARIATIONS OF BLOOD UREA OF FOUR SHORTHORN COWS DURING PREGNANCY.



■ = DRY PERIODS.

FIG 4. VARIATIONS OF BLOOD UREA OF FOUR FRIESIAN COWS DURING PREGNANCY.



■ = DRY PERIOD

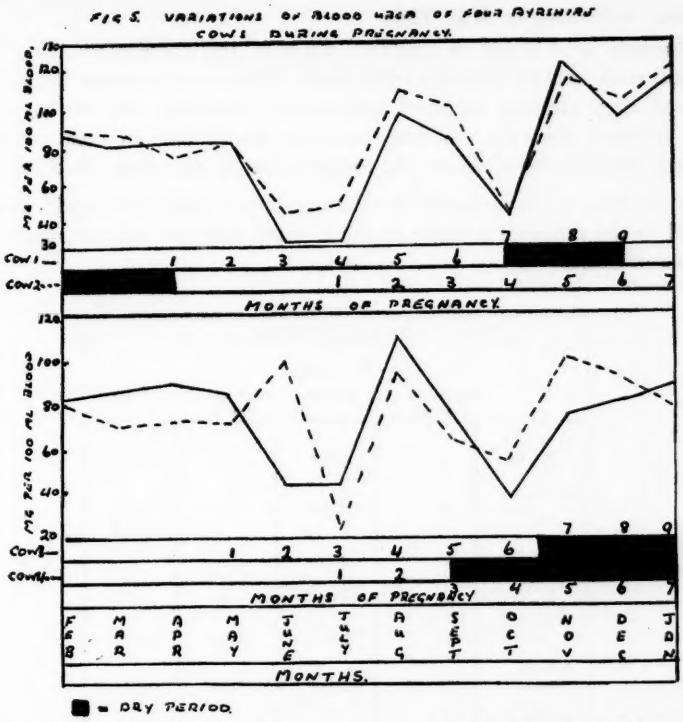


FIG 5. VARIATIONS OF BLOOD UREA OF FOUR AYRSHIRE COWS DURING PREGNANCY.

of the year were plotted and a downward curve during January, February and March was observed and this was followed by an upward curve from April until the end of the year.

Variations During Pregnancy.

Brown (1938) has determined the urea clearance in a number of pregnant women and found it somewhat greater than normal, indicating that the kidneys remove urea from the blood stream more efficiently, thus giving lower blood urea values. This also occurs in pregnant cattle during the first six months of pregnancy, but during the 7th, 8th and 9th month higher values were recorded.

Variations During Non-Pregnancy.

During the periods when the cows were not pregnant higher mean values were obtained than during pregnancy; this is shown on the right:—

BREED	PREGNANT CATTLE	NON-PREGNANT CATTLE
	Mean Figures mg per 100 ml of blood	
Guernseys	61.7.	73.8.
Shorthorns	75.8.	77.2.
Friesians	74.8.	81.5.
Ayrshires	75.1.	80.3.

Variations According to Milk Yield.

Attempts were made to ascertain whether any correlation exists between the blood urea content and the milk yield of the cow; marked fluctuations in the blood urea content occurred throughout lactation, and no inter-relation existed between the two. It was, however, found that in most of the cows the blood urea increased above the mean values as the cows dried off.

An analysis of variances of the data recorded shows the significance to be attached to the different months in adult cattle and the lack of significance in other observations.

Analysis of Variance

(a) Calves

	Degrees of Freedom	Variance	Variance Ratio	Significance
Different Animals	3	329.2	1.1	None
Different Ages	52	430.0	1.4	$\left\{ \begin{matrix} <0.05 \\ >0.01 \end{matrix} \right.$
Residual	156	300.0	-	-

(b) Adult Cattle.

Different Months	14	2291.2	4.60	<0.001
Between Breeds	3	1847.4	3.81	0.01
Within Breeds	12	305.0	<1	Nil
Residual	165	498.0	-	-

Summary

The urea content of the blood of calves between the ages of birth and 52 weeks and that of adult cattle from four dairy herds, namely, Guernseys, Shorthorns, Friesians and Ayrshires, have been studied.

With the adult cattle, consideration has been given to the variations which may exist between the different breeds, pregnant and non-pregnant animals, and the influence the milk yield might have on the actual amount of urea present in the blood of lactating animals.

Acknowledgments

The author thanks Dr. J. T. Abrams of the Department of Animal Husbandry for his help and advice with the statistical part of the observations.

REFERENCE

Brown, D. B. (1938): *J. Obstet. Gynæc., 45,* 786.

XUM

XUM

Œdematous endometritis, 45 days after intravaginal inoculation
(unserved heifer age 12 months)

(*Article by Millar, page* 309)

XUM

VIRAL INFERTILITY IN CATTLE

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WHILST studying herd-infertility problems, we have noted certain apparently infective forms of infertility which are not associated with the recognised genital diseases, brucellosis, trichomoniasis, and *Vibrio fetus* infection. The syndromes associated with these types of herd-infertility include the following: (a) Herd-infertility typified by a low conception-rate of progressive onset, post-coital genital discharges, irregularity of the œstrous cycles, and some incidence of early fœtal deaths and abortions. Vestibular vaginitis is commonly present, but not as a constant feature. The bull, or bulls, used in the herd suffer impaired semen production, the semen picture being that associated with sub-acute testicular inflammatory changes of a degenerative type. The available evidence is that the disease is transmitted only by coitus, the semen being infective. (b) This form of herd-infertility bears some superficial resemblance to that mentioned above. The mode of transmission is obscure, but, even if infection can be spread by coitus, it is not confined to the venereal route. This is evidenced by the fact that the appearance of symptoms of genital disease within the herd, including vaginitis and genital discharge, may be rapid and widespread. The onset is not related necessarily to coitus, so that maiden heifers, housed at some distance from the main herd, may show similar evidence of genital disease within a very few days after the appearance of symptoms in the adult females. An acute vestibular vaginitis is a constant feature of the syndrome, accompanied by a muco-purulent discharge of variable degree. The conception rate falls sharply.

We wish to refer in particular to the first type (a) of herd-infertility because we have been interested in it since 1953, and we have been able to devote more time to its experimental investigation.

Female

(a) *Pathogenesis.* When infective semen is deposited within the vagina, during mating by an infected bull, the uterine mucous membrane undergoes inflammatory changes of an œdematous type. Hyperactivity of the mucus-secreting glands of the cervix and uterine horns causes an increased discharge of mucus which is variably muco-purulent. The exact nature of this discharge is influenced to a considerable extent by the presence or absence of organisms of a potentially pyogenic nature. The normal flora of the female genital tract appears to become increased in virulence. These observations have been reproduced experimentally on a number of occasions, the normal flora of each of our experimental maiden heifers having been noted initially.

Gross uterine changes start within twelve to twenty-four hours after mating and are readily palpable at the fifteenth day. In experimental heifers, the nature of the uterus having been determined by previous examinations, the alterations of the endometrium may be palpable at the third day. During these early phases, the endometritis is of the nature of an œdematous thickening of the transverse endometrial folds (see Plate). In the absence of treatment, this endometritis

may continue for a period as long as seven months, but the acute stage is usually of shorter duration. We have not known these inflammatory changes to involve structures other than those of the uterine horns and cervix.

Preliminary, microscopic examinations of histological sections of the uteri of experimentally infected heifers have shown that the endometrium is thickened by changes in the epithelial and sub-epithelial cell layers. The cells of the epithelium and of the sub-epithelial stroma appear to undergo vacuolation. These vacuoles distend the affected cells and are found either surrounding the nucleus or displacing it to one side. The cellular condition appears to be one of hydropic degeneration. In some instances, vacuolation has been noted to be advanced almost to the stage of vesiculation. Bodies, somewhat spherical in shape and varying in size from a nucleolus to a small lymphocyte nucleus, have been noted. They are mainly basophilic in staining propensity, but some stain a reddish colour with the methods of Goodpasture and Lendrum. We do not know whether these are true inclusion bodies or merely nuclear fragments.

(b) *Symptoms.* An increased flow of genital discharge is noted within forty-eight hours after service, usually with a variable addition of pus cells. The mucus may contain flakes of purulent material or, even, be uniformly purulent. Vestibular vaginitis may occur, but not as a constant feature. Within three days after infection, pus flakes may be found adhering to the vaginal mucous membrane within the vestibule or in the folds of the clitoris. The œstrous cycle tends to become irregular. In females which have failed to conceive following infection, the cycles tend to shorten to eighteen days. Others, which may have been fertilised, may return at about twenty-four to twenty-eight days. Uterine palpation reveals slightly increased tension and distension of the uterine horns so that they present a markedly " rounded " section, particularly near the tubal extremities

The lowered fertility associated with infection does not appear to be of a lasting nature, fertility usually tending to be recovered within about five months. On the other hand, at least one of our experimental heifers remains infertile after an interval of seven months following infection.

Male

(a) *Pathogenesis.* We know very little about this aspect, partly because the affected bulls which we have found in herds have recovered following treatment, so that we have not been able to conduct a post-mortem examination. Sufficient indirect evidence is available, however, to suggest that the spermatogenic tubules of the testicles temporarily undergo inflammatory changes and suffer loss of function. In the intermediate stages, before the function of spermatogenesis is completely lost by a tubule, it produces non-viable spermatozoa, some of which show morphological evidence of interference with development during the final haploid phase of cell-differentiation. As the seminal plasma appears to be just as infective as the whole ejaculate, the seminal vesicles may harbour infection. Clinically, there is little evidence that the seminal vesicles suffer inflammatory changes.

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(b) *Symptoms.* At this stage we can discuss only the evidence shown by bulls which have become infected naturally. In the early stages, the testicles become more tense and slightly hypertrophied. They are sensitive on palpation. Apparently the epididymes do not alter. After a period, which is variable, but may be as prolonged as six months, the testicles soften and show evidence of atrophy.

The semen picture varies with the length of time which has elapsed since infection, with the age of the animal, and with the presence, or absence, of potentially pathogenic, pus-forming organisms. To some extent, also, it is influenced by the amount of work demanded of the bull. In the early phases of inflammatory change there is a slight tendency for the semen production to be enhanced, not only in volume but in spermatozoon concentration. This phase is followed by a decrease in the volume of the ejaculate and by a greater fall in the spermatozoon concentration. At the same time, the percentage of dead and degenerated spermatozoa is increased, with a resulting lowering of the motility picture and of the viability of the sample. Abnormalities of morphology also appear associated with tubular inflammatory changes. These spermatozoon abnormalities are such as to cause the middle portion of the tail and the tail filament to form a tight coil either at the base of the head, sometimes enclosing it, or at the distal end of the middle portion. There may also be a noticeable presence of protoplasmic droplets in the seminal plasma, some adherent to the spermatozoa. In only one instance did the ejaculate from an infected bull fail to contain any sperms. These ejaculates contained masses of pus-cells over a period of months.

A notable feature of the above semen defects is that they have been eliminated following treatment. This was so even in the extreme instance mentioned above. After treatment, the relevant bull could not have been judged, by any standards, to have suffered genital malfunction.

Even after an affected bull has reached the stage of producing abnormal ejaculates his keenness to serve remains undiminished. In the early stages of infection his libido may be enhanced. There is a natural tendency, however, for affected bulls to be overworked in herds suffering infertility, so that some degree of slowness may become apparent ultimately.

Herd Picture

As always happens with herd outbreaks of genital disease of coital significance, the fertility of the herd can be shown to have been satisfactory until the introduction of one or more animals, of either sex, from another herd. Some time must elapse, however, before conception failures attract attention. It may be that the occurrence of post-coital discharges will have been noted in individual females before a lowered conception-rate causes anxiety. The œstrous cycles of infected, non-pregnant females tend to become irregular, and individuals which had been thought to be pregnant "return" unexpectedly. After infection has been present for some considerable time, when the fertility picture possibly appears to be improving, the onset of abortions, at periods varying widely from ten weeks

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up to eight months, may occur. The fœtuses, membranes and materials collected from the dams will fail to reveal the presence of any known pathogenic organism.

A feature commonly noted is an œdematous condition of the fœtal membranes. The early fœtus, of about three months, may be born in intact membranes and the chorio-allantoic fluids have the consistency of a clear, yellowish jelly. This œdema may be associated closely with that of the uterine endometrium, but we have not found any aborted fœtuses to be œdematous. There is some evidence to suggest that the occurrence of undetected early fœtal deaths may elicit a clinical picture of irregular and late returns to service. When infection has been present in a herd for some considerable period, uterine involution may be impaired and associated with a post-parturient leucorrhœa.

Diagnosis.

We have investigated a number of possible laboratory techniques, so far without success. These methods will be discussed with the experimental findings. We can judge only on the presence of the symptoms mentioned above and the negative laboratory findings relative to known diseases.

Prophylaxis.

The introduction of infection into unaffected herds can be prevented only by maintaining them in a self-contained condition. There are numerous ways by which infection may be introduced, including the purchase of females which have been mated, whether pregnant or not, the introduction of a used bull or of semen collected from an infected bull. It might be that the potential risk, associated with the introduction of semen, could be diminished by the addition of antibiotics to semen before distribution but this, at present, can be only a matter of conjecture. The risks inherent in the hiring or lending of bulls are obvious.

Control.

When this type of infertility has been found in a herd, the measures of control must be influenced by the extent to which disease has spread already by coitus. Until this can be determined by clinical evidence and by a study of the service data, all services should be stopped. In this way, if the spread has not become too great, it may be found that some females have not been exposed to infection, having either been pregnant at the time of introduction or, as in the case of maiden heifers, unserved. When more than one bull is in use, it may be that only a particular group of females and one bull are involved. The indiscriminate use of infected bulls will soon involve all the mated animals in a herd.

As the semen itself is infective, the use of an A.I. technique employing infected bulls will give no protection to unexposed females. For this reason, such females can be mated only to clean bulls, although infected females, after treatment, can be inseminated from clean bulls, or inseminated from treated bulls which have recovered. Natural mating may be attended by some risk of re-infection to the bull from a treated, unrecovered female. We do not know whether immunity can be acquired or, if an immunity does develop, how strong it can be when challenged by re-exposure to infection.

Treatment.

(a) *Female.* The symptoms associated with the endometritis, including infertility, have yielded fairly readily to the intra-uterine use of streptomycin in herds studied in the field. It may be that other antibiotics would have proved of equal or greater value in treatment, but we have no experience of their use. In the presence of a potentially pathogenic organism, such as *Corynebacterium pyogenes,* and when the genital discharge becomes very copious and purulent, it appears that uterine irrigation with acriflavine and glycerin acts as a cleansing agent, allowing the antibiotic to be used more effectively.

(b) *Male.* The only method of treatment used has been the repeated intramuscular injection of streptomycin and penicillin. In all, ten bulls have been treated in this way. They recovered fertility, sometimes from a condition of virtual sterility, sufficient to be used either by artificial insemination or by natural mating. Of possibly greater significance, the use of these bulls has not been followed by the reappearance of genital disease in the females mated to them.

Experimental Observations.

The materials originally obtained from the field were samples of genital mucus and semen from infertile animals in affected herds. These samples were kept in storage at —20° C. Following centrifugation at 10,000 r.p.m. for one hour and subsequent passage through a filter such as Seitz E.K. or Gradocol membrane, with a pore diameter of 0.59μ, these materials were diluted with beef-heart-infusion broth; the dilution rate varying from one volume in ten to one in twenty. Tests carried out on these materials before and after they were used for inoculation, showed that they contained no bacteria.

The intravenous inoculation of unused bulls and heifers failed to induce evidence either of systemic reaction or of genital inflammation. Preliminary experiments were also carried out to determine whether any of the processes associated with the intra-vaginal inoculation of heifers would elicit signs of vaginitis or other genital abnormality. It was found that neither the introduction of a sterile glass pipette nor the deposition of bacteria-free fluids, such as beef-heart infusion broth and filtrates of mucus or semen from young unused animals, would stimulate any reaction. The addition to the inoculum of 10 per cent bacteria-free filtrate of the field materials, however, showed a definite response after intravaginal inoculation. Within twenty-four hours, abnormal genital discharge was readily noted, sometimes accompanied by the onset of vestibular vaginitis. It appeared that the seminal plasma was a more active source than mucus, being active in higher dilution ranges, but the mucus was virulent at 1 :20. Control heifers were housed closely with reacting, inoculated heifers and showed no evidence of genital abnormality. This finding agreed with the observations made in herds, in which served heifers were confined in loose-boxes with unmated heifers, and infected cows in the sheds were standing alongside unserved animals. When the physical characteristics of the uterus of each maiden heifer had been determined by previous examinations, the onset of endometritis was just detectable on the third day, being well established and readily palpable by the sixteenth day. Flakes of purulent material could be seen, when the lips

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of the vulva were parted, adhering to the vaginal mucous membrane of the vestibule. Some degree of slight vaginitis developed. It was found that infection could be passaged readily from one heifer to another by the introduction of filtered genital mucus.

Following the infection of a group of experimental heifers either by such methods or by employing a small pad of sterile absorbent cotton wool for the conveyance of the filtrate into the vagina, they were mated to two young, unused bulls. Within two months, these bulls were allowed to serve clean maiden heifers which showed evidence of genital reaction within forty-eight hours, accompanied by a muco-purulent discharge. These findings indicated that mechanical transmission at least was possible and that the response was similar to that occurring under field conditions and in our inoculated heifers. Within the past six months, the semen production of only one of these bulls has shown deterioration, but, for a period, both were yielding samples above the average for their age and breed. Later services showed that inflammatory changes could be induced in maiden heifers, with purulent genital discharge, without necessarily being sufficiently severe to prevent conception.

Following the intra-vaginal inoculation of she-goats, the naturally heavy cell-content of the genital mucus in this species tended to mask any visible evidence of possible reaction. However, post-mortem examinations carried out at about the sixteenth day showed that the uterine reaction was greater than that found in heifers, the endometrium being more grossly altered and œdematous than that of affected heifers. Unfortunately, such changes were not palpable during life.

Embryonated hens' eggs were divided into two groups, according to the route employed and inoculated with bacteria-free genital mucus, diluted 1 : 20 with beef-heart infusion broth. In one group, the inoculum was deposited on the chorio-allantoic membrane; in the other, the inoculum was deposited in the yolk-sac. There was no mortality of the embryos and the eggs remained bacteria-free. In the first group there was marked thickening of the chorio-allantoic membrane. In the second group the yolk was much coagulated. From these, materials were harvested, emulsified, diluted, filtered and subjected to sterility tests in preparation for further egg passage. Such passages were maintained to the twelfth C.A.M. and to the eighth Y.S.

One heifer was inoculated intravaginally with a diluted emulsion of third chorio-allantoic membrane passage and one with third yolk-sac passage. Both inocula were active in reproducing the œdematous endometritis and genital discharge, but the C.A.M. material appeared more potent in inducing the vestibular vaginitis. This is not considered to be of any significance. As a further step in transmission, both of these heifers being destroyed, a portion of œdematous mucus membrane was emulsified, tested for sterility, filtered and diluted with beef-heart infusion broth. Two millilitres of this inoculum was deposited within the vagina of a further heifer which reacted by the onset, within twenty-four hours, of genital discharge and by the development of the œdematous endometritis.

Although the appearance of vaginitis is frequently mentioned in this investigation we do not consider that it necessarily has any significant relationship

to fertility. We did wonder, however, whether our original material contained more than one filterable agent. Although vaginitis is commonly present in these infertile females, there are many which do not suffer vaginitis. If two infective viruses are present in such a herd, they may be transmitted together at coitus but only the less evident may be of real importance.

One hundred and eight mice were inoculated, by various routes, with varying dilutions of bacteria-free egg passage material. Samples of these materials were known to be capable of inducing genital disturbances in heifers. There was no evidence of ability to produce lesions or death in the mouse.

Complement Fixation Tests.

Blood sera from ten heifers, collected at varying periods up to one hundred and four days after inoculation, serum from one in-contact heifer, and serum from one healthy maiden heifer, were tested as a preliminary measure. At a a later date a further series of blood tests were carried out, using four groups of pooled sera representing uninoculated heifers as a control group, and three varying periods after inoculation (9 to 18, 32 to 46, and 100 to 104 days). On a third occasion, pooled infected and pooled normal bovine sera were titrated against varying dilutions of infected and normal antigens, prepared from bovine uteri, in the presence of varying complement dilutions. Although the findings were negative such as to indicate that the blood of infected animals might not be invaded, and that the infection might be of purely local significance, we have no conclusive evidence on this point.

There is a need for the development of a rapid, cheap and accurate laboratory method of diagnosis, not only with regard to herd infertility problems —which can be relieved by control and treatment—but with special reference to individual animals such as bulls which may transmit infection to clean herds, either by natural mating or by artificial insemination. This is the aspect of the problem which is causing the greatest concern.

Summary

A syndrome of herd infertility in cattle has been described. It appears to be associated with the venereal transmission of a filterable virus which, although capable of inducing an endometritis of specific type, may further impair fertility by enhancing the virulence of potentially pathogenic organisms already present in the genital tract.

No attempt has been made to ascertain the incidence of this herd syndrome, but it is appreciated that clinically it shows aspects commonly associated with *Vibrio fœtus* infection. It is known, also, that some herd problems, initially expected to be linked to Vibriosis, fail on further investigation to reveal the presence either of *V. fœtus* or of any other known pathogen.

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ABSTRACT OF LITERATURE

CLINICAL EVALUATION OF A NEW SYSTEMIC HEMOSTATIC AGENT, by C. W. Cromley, D.V.M. *Veterinary Medicine,* June, 1955.

THIS report concerns six months' successful trials of a new drug, "klot," which is claimed to be rapidly effective in the control of hæmorrhage in large and small animals. Success is mentioned in Cæsarean Section, Hæmatomas, Damaged Horns, Bloody Milk and Surgical Procedures in Small Animals. The action of the drug occurs at the point of trauma and also accelerates clotting time, arrests internal bleeding, facilitating operative procedures. No interference with anæsthesia or other drugs was noticed, but in one instance after tonsillectomy on a dog, "klot" failed to control bleeding in the tonsil pits. The author suggests this may have been due to the fact that intensive antibiotic and sulphonamide therapy had been administered for a period of 10 days prior to the operation to arrest infection.

ITEM OF VETERINARY INTEREST

PYE LTD., of Cambridge, state that their radio-telephone system has been put to profitable use in veterinary practice. They quote a case in the Midlands where two surgeons in partnership use this form of communication and say the ability to call one partner while still on his rounds has saved time and expense in duplication of journeys to neighbouring districts and speeds up attendance to emergency calls. Dispensing instructions in the same way can be passed back by the attending surgeon for immediate preparation at the surgery. We can appreciate the particular value of this system to veterinary practitioners who perform their duties frequently out of touch with ordinary telephone communications.

ECHO FROM THE PAST

(*From the* VETERINARY JOURNAL, *July,* 1880)

HORSE PLAGUE IN CENTRAL ASIA

THE *Moscow Gazette* of a recent date contains a paragraph from the official *Semipalatinsk Vedomosti,* stating that a terrible horse plague has broken out in the province near the Chinese frontier. In the Pavlodar and Karkaralin districts not a horse remains alive. The officials are unable to collect information respecting the losses, because no horses are left for them to ride upon. The postal service on the post-roads has been stopped by the death of the post-horses, and in the villages the peasants have had to cease farming. The disease is believed to be the same as that which has been raging in Chinese Mongolia since 1875, and which has yearly been advancing towards Russia.

ERRATUM

THE illustrations accompanying the article by Mr. P. G. Millar on page 258 of June issue of the Journal have been transposed. That marked as Figure 1 should be Figure 2, and vice versa.